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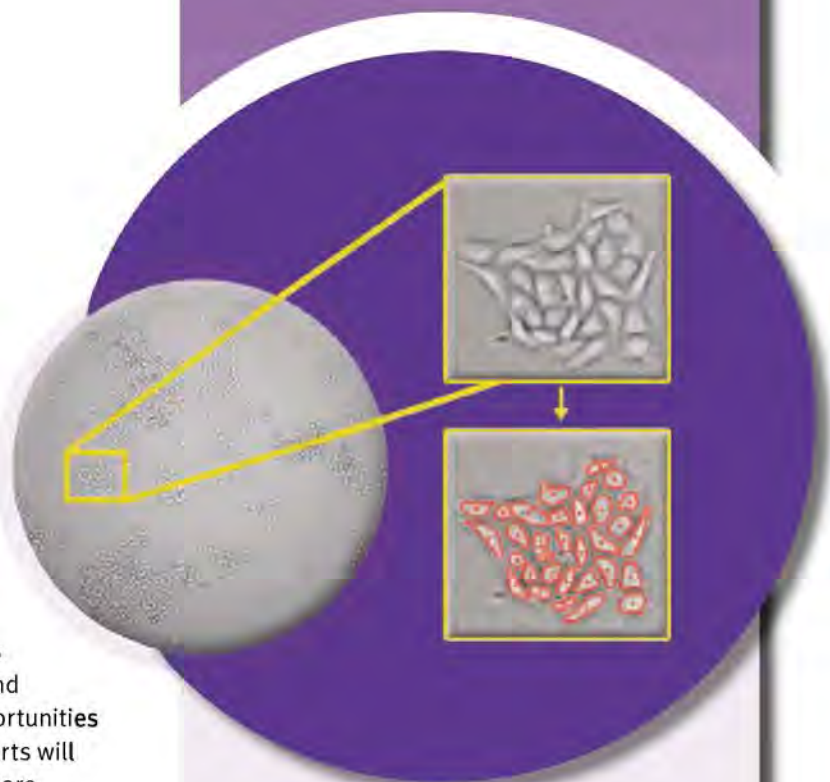
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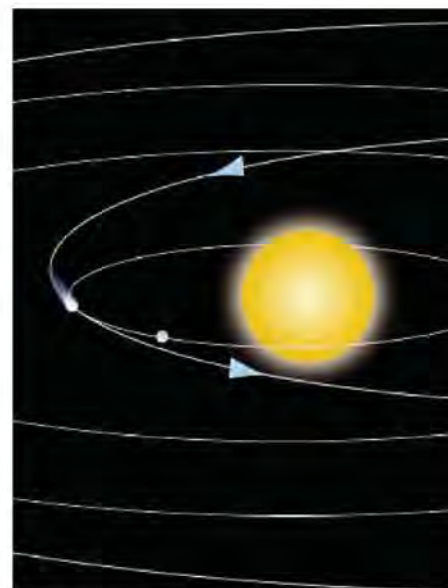
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Ants live together in highly complex societies, often sacrificing individual gain to achieve common goals such as forming a living bridge. Such cooperation makes evolutionary sense because the ants are all related, as detailed in this month's Origins essay on page 1196. The essay and two Reports on pages 1269 and 1272 address how cooperation evolves in unrelated individuals and even between species.

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Sample & Assay Technologies

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- 1234 **Reassessing the Source of Long-Period Comets**
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- 1254 **Regulation of Histone Acetylation in the Nucleus by Sphingosine-1-Phosphate**
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- 1258 **Perineuronal Nets Protect Fear Memories from Erasure**
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- 1265 **Recruitment of Antigen-Specific CD8⁺ T Cells in Response to Infection Is Markedly Efficient**
J. W. J. van Heijst et al.
Lymphocyte proliferation, more than recruitment to the site of an infection, determines the success of the immune response.
- 1269 **Differential Sensitivity to Human Communication in Dogs, Wolves, and Human Infants**
J. Topál et al.
Social interactions with humans govern the way dogs and children learn, but wolves learn by focusing on objects.
- 1272 **Positive Interactions Promote Public Cooperation**
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Reward is as good as punishment to promote cooperation, costs less, and increases the share out of resources up for grabs.
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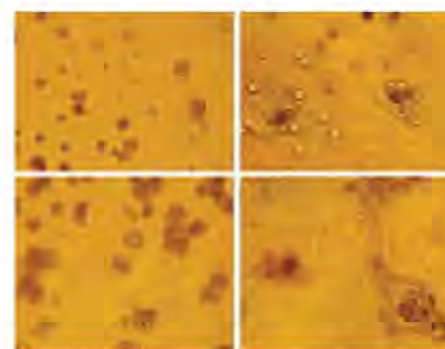
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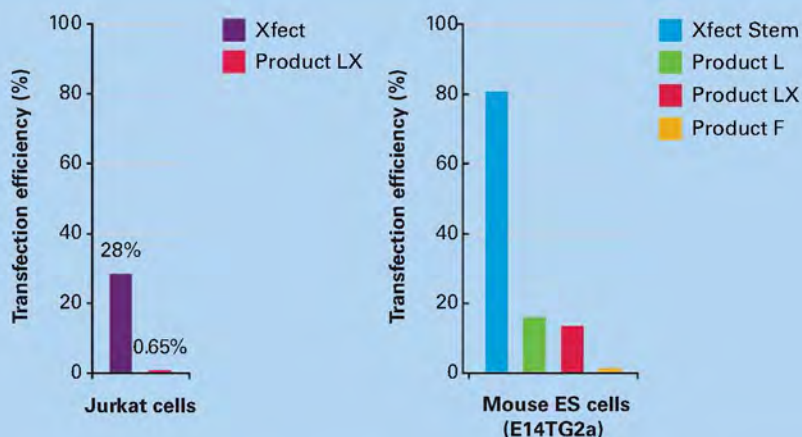


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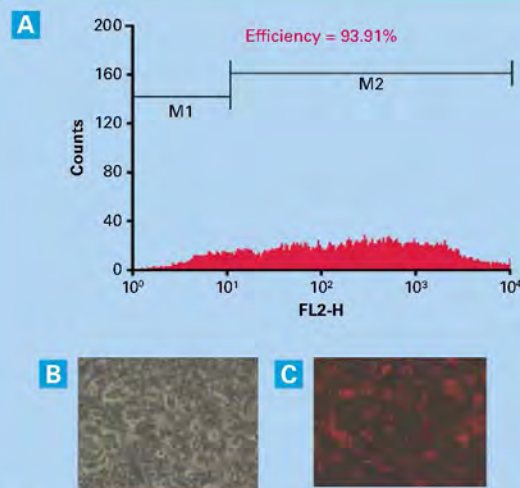
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Dirac Strings and Magnetic Monopoles in Spin Ice $\text{Dy}_2\text{Ti}_2\text{O}_7$

D. J. P. Morris et al.

10.1126/science.1178868

Magnetic Coulomb Phase in the Spin Ice $\text{Ho}_2\text{Ti}_2\text{O}_7$

T. Fennell et al.

Neutron scattering measurements on two spin-ice compounds show evidence for magnetic monopoles.
10.1126/science.1177582

Unbiased Reconstruction of a Mammalian Transcriptional Network Mediating Pathogen Responses

I. Amit et al.

Inflammatory and antiviral programs in dendritic cells are controlled and tuned by a network of regulators.
10.1126/science.1179050

Broad and Potent Neutralizing Antibodies from an African Donor Reveal a New HIV-1 Vaccine Target

L. M. Walker et al.

High-throughput screening has revealed two new broadly neutralizing antibodies from a clade-A-infected donor in Africa.
10.1126/science.1178746

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Genetic Discontinuity Between Local Hunter-Gatherers and Central Europe's First Farmers

B. Bramanti et al.

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10.1126/science.1176869

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Smoothered Mutation Confers Resistance to a Hedgehog Pathway Inhibitor in Medulloblastoma

R. L. Yauch et al.

A mutation that prevents binding of a promising drug lead to its target protein confers resistance in a human brain tumor.
10.1126/science.1179386

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Comment on "Energy Uptake and Allocation During Ontogeny"

A. M. Makarieva et al.

full text at www.sciencemag.org/cgi/content/full/325/5945/1206-a

Comment on "Energy Uptake and Allocation During Ontogeny"

T. Sousa et al.

full text at www.sciencemag.org/cgi/content/full/325/5945/1206-b

Response to Comments on "Energy Uptake and Allocation During Ontogeny"

W. Zuo et al.

full text at www.sciencemag.org/cgi/content/full/325/5945/1206-c

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RESEARCH ARTICLE: IL-1 Induces IGF-Dependent Epithelial Proliferation in Prostate Development and Reactive Hyperplasia

T. J. Jerde and W. Bushman

A classically inflammatory mediator, interleukin-1, also functions during development in the prostate.

PERSPECTIVE: Tumor Suppression by LKB1—SIK-ness Prevents Metastasis

R. J. Shaw

Salt-inducible kinase 1 is activated by LKB1 to promote anoikis, thereby blocking metastasis.

REVIEW: Stress at the Synapse—Signal Transduction Mechanisms of Adrenal Steroids at Neuronal Membranes

E. M. Prager and L. R. Johnson

Adrenal steroids regulate neuronal excitability by altering ion channel conductance or gene transcription.

PROTOCOL: Analysis of Signaling Events by Dynamic Phosphoflow Cytometry

G. Firaguary and J. A. Nunès

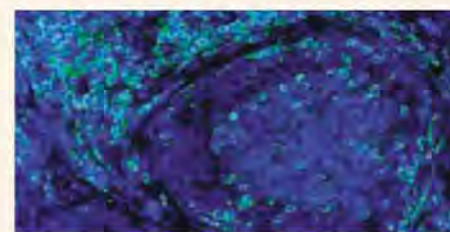
State-specific antibodies combined with flow cytometry detect the activity of signaling pathways.

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T cells in an inflamed prostate.

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I. S. Levine

September brings inevitable but manageable stress for academic scientists.

Taken for Granted: A 'Natural' Step?

B. L. Benderly

Postdocs join their bosses' union at Rutgers University.

Choosing the Less Traveled Road

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Molecular biologist Lars Jansen owes his success to not taking the easy path.

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A History of Beginnings

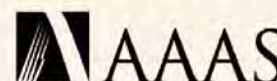
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Science Policy News and Analysis

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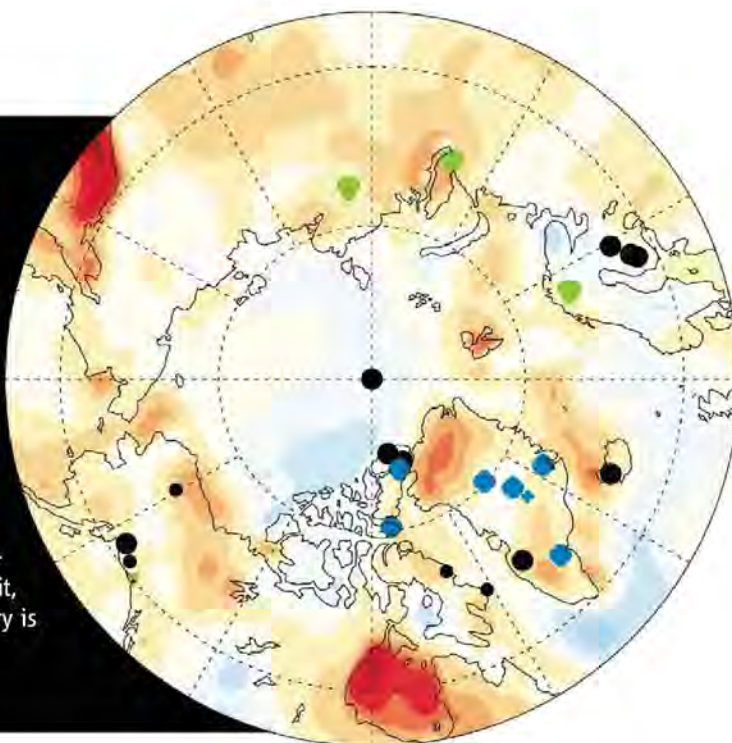


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EDITED BY CAROLINE ASH

Climate Reversal >>

The climate and environment of the Arctic have changed drastically over the short course of modern observation. **Kaufman *et al.*** (p. 1236) synthesized 2000 years of proxy data from lakes above 60° N latitude with complementary ice core and tree ring records, to create a paleoclimate reconstruction for the Arctic with a 10-year resolution. A gradual cooling trend at the start of the record had reversed by the beginning of the 20th century, when temperatures began to increase rapidly. The long-term cooling of the Arctic is consistent with a reduction in summer solar insolation caused by changes in Earth's orbit, while the rapid and large warming of the past century is consistent with the human-caused warming.

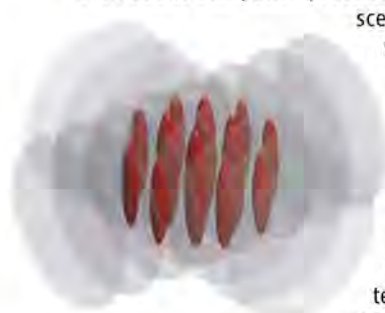


Stability Through Confinement

With the ability to vary experimental parameters—including particle density, geometry, interaction strength, and sign—cold atoms trapped in optical lattices are ideal test systems to probe many-body quantum physics. However, due to dissipation processes most of the

scenarios studied to date have necessarily looked at ground state phases. **Haller *et al.*** (p. 1224) describe a technique in which confinement

of the atoms to low dimensions, using a confinement-induced resonance, can stabilize excited states with tunable interactions. The ability to create these metastable states will allow a much wider range of quantum systems to be explored.



Massive White Dwarf

White dwarfs are the remnants of stars like the Sun, which blow their outer layers away and compress into dense cores as they end their nuclear-burning lives. **Mereghetti *et al.*** (p. 1222) observed an x-ray eclipse of a peculiar system comprising a white dwarf (which is also a fast pulsating x-ray source) and a subdwarf star (a star that lost its outer layers before nuclear fusion was extinguished in its

core). Based on dynamical considerations and the presence of x-ray pulsations, the mass of both stars has been estimated. The white dwarf has a mass at least 1.2 that of the Sun, which is close to the limit of gravitational stability for this class of star. These results provide constraints on the poorly understood process that led to the formation of our solar system.

Inside Oort

Long-period comets are thought to come from the outermost region of the solar system, the Oort Cloud, where a large number of icy bodies orbit the Sun. Outer Oort Cloud bodies are more likely to penetrate the inner planetary region of the solar system as comets, because they experience stronger external gravitational perturbations. Inner Oort Cloud bodies, by contrast, are thought to be ejected before they reach observable orbits. Using numerical simulations that followed the orbital histories of bodies in the inner Oort Cloud, **Kaib and Quinn** (p. 1234, published online 30 July 2009; see the Perspective by **Duncan**) identified a dynamical pathway that allows comets in the inner Oort Cloud to move to the outer Oort Cloud, where they can be perturbed and enter the visible region in great numbers. According to this mechanism, the amount of mass in this region is consistent with the material needed to form the giant planets.

Epigenetic Signals

The lipid sphingosine-1-phosphate (S1P) is a signaling molecule that binds to receptors on the cell surface to initiate biochemical changes

that control a range of biological processes from growth and survival to immune reactions. **Hait *et al.*** (p. 1254) report that S1P can also function by direct binding to the nuclear enzymes, histone deacetylases (HDACs) 1 and 2. The enzyme that generates S1P, sphingosine kinase 2 (ShpK2) is present in the nucleus in complexes with HDAC1 and HDAC2. Generation of S1P and its binding to HDACs inhibited deacetylation of histone. Such histone modification is an epigenetic mechanism that controls gene transcription. Thus, generation of S1P in the nucleus appears to be a signaling mechanism by which cells can control gene expression in response to various stimuli.

Adult Fears

Why are fear memories almost impossible to get rid of—even with extensive extinction training? Animal studies have shown that the efficacy of extinction learning depends on age. Fear memories in young animals can be permanently erased, but in adults they can be easily recovered after extinction training. Perineuronal nets, the highly organized form of extracellular matrix around inhibitory neurons, mediate the shift from juvenile to adult forms of learning in sensory systems. **Gogolla *et al.*** (p. 1258; see the Perspective by **Pizzorusso**) have discovered that the formation of perineuronal nets in the amygdala coincides with the developmental shift in the ability to erase fear memories by extinction. Removal of perineuronal nets in adult animals re-enabled the erasure of fear memories. Thus, in adults it appears that fear memories are actively protected from erasure by the perineuronal nets.

CREDITS (TOP TO BOTTOM): KAUFMAN ET AL.; HALLER ET AL.

Adaptive Limits

Species adapt to a changing environment as a result of selection acting on current genetic variation. However, the degree of variation underlying traits that can respond to selection is unclear. **Kellermann *et al.*** (p. 1244; see the Perspective by **Merilä**) investigated the degree of genetic variation available to fruit flies for cold and desiccation tolerance. Species from the tropics tended to have low variability for these traits, while flies from more temperate climates showed higher levels of variation. However, overall genetic variability did not differ, suggesting that the tropical species lacked the alleles that confer tolerance to these environmental extremes and restrict their potential range.

Preparation for Cell Wars

When T cells encounter an infection, they proliferate to create a larger army to fight the invader. The overall magnitude of the T cell response depends on the severity of infection and is determined by the number of T cells of a particular antigen specificity that are initially recruited, as well as the magnitude of the proliferative response. The extent to which these two components contribute to the response is unknown. By using DNA barcoding to track the responses of individual T cells, **van Heijst *et al.*** (p. 1265) showed that the recruitment of T cells of a particular antigen specificity is similar and nearly complete, but that the extent of the proliferative response differed, and this determined the overall magnitude of the T cell response.

Fascin-Actin Rab Bristles

Rab proteins have diverse functions in directing intracellular traffic and may also affect development. **Zhang *et al.*** (p. 1250) show that during *Drosophila* development Rab35 influences the development of bristles, neuromuscular structures built upon bundled actin. Rab35 also caused massive actin-rich filopodia protrusions from cultured cells. Activated Rab35 interacted directly with fascin, an actin filament bundling protein, to colocalize near the plasma membrane. When Rab35 was engineered to interact with the surface of mitochondria, it stimulated localized actin assembly in a fascin-dependent manner. Thus, fascin is a Rab35 effector protein that links membrane trafficking regulation to cytoskeleton assembly during development.



Boxing Clever?

Piaget showed that 10-month-old infants will persist in looking for a toy in box A, where it has been placed several times, even after having been shown that it has been moved to box B, whereas 12-month-old infants do not. This phenomenon marks a developmental milestone in human infant cognition that **Topál *et al.*** (p. 1269; see the Perspective by **Tomasello and Kaminski** and the news story by **Pennisi**) explored in a remarkable series of comparative tests. The results support the view that infants and adult dogs will both persevere in searching erroneously in box A because they regard the placement of the toy by a human experimenter as a social teaching event. By contrast, wolves rapidly learn correctly to search box B. They also observed that infants are able to generalize and thus still persevere when one experimenter places the toy in box A and a second then places the toy in box B. Dogs, however, display episodic learning, and a second experimenter reduces their searching choice to chance.

Carrots Are Better Than Sticks

The challenge of dealing with freeloaders—who benefit from a common good but refuse to pay their “fair share” of the costs—has often been met in theoretical and laboratory studies by sanctioning costly punishment, in which contributors pay a portion of their benefit so that freeloaders lose theirs. **Rand *et al.*** (p. 1272; see the news story by **Pennisi** and the cover) added a private interaction session after each round of the public goods game during which participants were allowed to reward or punish other members of their group. The outcome showed that reward was as effective as punishment in maintaining a cooperative mindset, and doing so via rewarding interactions allowed the entire group to prosper because less is lost to the costs of punishing.

CREDIT: ZHANG ET AL.

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Galvanizing Science Departments

COUNTLESS REPORTS HAVE STRESSED THE ECONOMIC AND BROAD SOCIETAL BENEFITS TO BE GAINED from improved science, technology, engineering, and math (STEM) education for all students. At the higher education level, there is extensive evidence that innovative teaching methods improve student learning and are practical to implement. Yet these methods remain at the periphery, and the traditional lecture model continues to dominate, particularly at large research universities. This fact poses a major problem for improving science education at all levels, because these institutions generally set the norms for how to teach science and what it means to learn science. To effectively change STEM education at the university level, a majority of the faculty in a given university department must become collectively engaged in implementing new curricula and teaching methods. In other words, an entire department must be the unit of change.

A possible model for achieving such departmental change at a research university* is being tested at the University of Colorado (CU) and the University of British Columbia (UBC), with promising initial results. This model introduces evidence-based STEM teaching methods into most undergraduate science courses. Now in the fourth year of a 6-year project at CU, over 60% of the faculty in three major science departments have changed their teaching approaches, affecting 80% of each department's student credit hours. The changes include adding explicit learning goals for a course that are expressed in terms of demonstrated student capabilities, and using teaching methods proven to get students more mentally engaged. For example, collaborative activities have students practice scientific thinking and problem-solving with constructive feedback, and pre- and post-course testing measures what students actually learn in a course. These pre-post tests have shown that substantial learning is being achieved and that there is clear improvement where appropriate comparisons with traditional teaching methods exist. These test results also guide yearly improvements. Within departments, the teaching conversation has shifted from the standard concerns about topic coverage to a new focus on how students learn, pedagogy issues, and evidence of learning.

The model uses substantial one-time university funds to change faculty practices and produce carefully designed courses that will be reused in the future with no additional cost and considerable savings in time. To compete for funds of approximately \$1 million, distributed over 6 years, a department must lay out a process for establishing well-defined learning goals, rigorous assessment of learning, and implementation and testing of improved teaching methods for each of its core undergraduate courses. Producing such an integrative plan requires intense discussion among the faculty that is focused on the department's undergraduate educational goals and practices. Usually, such a discussion is unprecedented. Thus far, all of the departments at CU and UBC receiving such funding have hired department-based science education specialists (SESs) to help facilitate the change process. A successful SES has mastered the particular STEM discipline, has knowledge of educational and cognitive psychology research, is familiar with proven teaching methods, and possesses diplomatic skills. The SES works with faculty to develop and implement the components of improved teaching in their courses, and ensures that course materials are passed to subsequent instructors in a readily useable form.

The goal at the end of the 6 years is to have most of the faculty embrace this approach to learning and to have transformed most of a department's courses. Moreover, there will be departmental expectations that faculty will use these materials and methods, thereby saving time, increasing effectiveness, and ensuring sustainability without ongoing funding. The faculty members involved say that their biggest rewards are experiencing how much more engaged their students are and the stimulation they receive from discussing teaching with colleagues as a scholarly activity. This kind of change in departmental culture is surely what is needed to improve STEM education at the higher education level, no matter what the model.

— Carl Wieman

10.1126/science.1180564



*www.cwsei.ubc.ca

ECOLOGY

Fishing Down a Halo

Breeding seabirds are among the many animals that return to a central location after obtaining food. Such central-place foragers are expected to favor prey captured nearby. This preference could deplete prey in the area around the colony (forming a "Storer-Ashmole's halo") through the removal of benthic species or the departure of mobile pelagic species. For marine predators, tests of this hypothesis must also consider water depth, which might have a greater impact than distance on foraging efficiency. Breeding thick-billed murres (*Uria lomvia*) usually return to their chick with a single easily identifiable item of food, and the birds are large enough to carry time-depth recorders that have little effect on their behavior. Thus, by monitoring prey deliveries of chick-rearing murres equipped with recorders, Elliott *et al.* were able to attribute individual prey items to specific dives. Drawing on data gathered over many years at a colony numbering roughly 100,000 individuals on Coats Island in northern Hudson Bay, they restricted their analyses to the interval of constant energy-delivery rates to chicks. Prey mass, and hence energy content, increased with distance from the colony, and dive depths became shallower as distance increased. As the breeding season progressed, murres flew farther for a given prey. Within each season, birds fished down the food web: taking larger fish first, then smaller fish, and even smaller invertebrate prey last. The prey depletion demonstrated by these four foraging patterns can lead to density-dependent population regulation and the life-history strategies of delayed maturity, low fecundity, and high adult survival found in many seabirds. — SJS

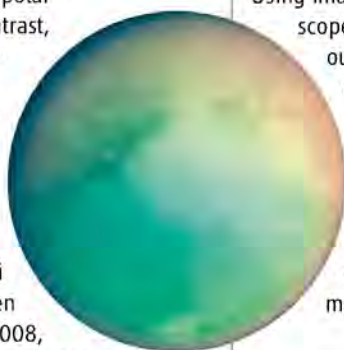
Auk 126, 613 (2009).



ASTRONOMY

Clouds in the Tropics

Previous observations of Saturn's largest moon showed methane clouds at high southern latitudes, where summer prevailed, and ethane clouds in the winter north polar region. The tropics, by contrast, tended to appear virtually cloudless. Observations from Cassini and ground-based telescopes have now revealed a greater recent incidence of clouds in Titan's tropical atmosphere. Using Cassini observations taken between 3 July 2004 and 28 May 2008, Griffith *et al.* detected the presence of five tropical clouds with characteristics similar to those of the summer polar methane clouds. This increasing cloud presence provides evidence for the beginning of an overturn in Titan's atmospheric circulation; Titan's pole-to-pole circulation is expected to reverse near the equinox, which occurred this August, inducing the polar clouds to swap hemispheres.



High-latitude clouds can reach altitudes of 45 km, but the Cassini observations show that tropical clouds are confined to altitudes below 26 km. Although this finding points to a dry and stable tropical climate, another recent study implicates rather different weather patterns.

Using images from two ground-based telescopes, Schaller *et al.* report large cloud outbursts starting at southern mid-latitudes in April 2008, triggering cloud activity near the south pole and the equator of Titan over the next 3 weeks. The cause of this outburst is unknown, though it is thought to be associated with stormy weather conditions and substantial amounts of methane rain. — MJC

Astrophys. J. 702, L105 (2009);

Nature 460, 873 (2009).

BIOPHYSICS

Swimming Through Mucus

The stomach is an acidic environment of pH ~ 2 as anyone who has suffered a bout of emesis can testify. So how does *Helicobacter pylori*, which is not an acidophilic bacterium, survive? First, it secretes

the enzyme urease, which hydrolyzes urea to produce ammonia. This helps to neutralize the ambient hydrochloric acid, at least locally. Another stratagem is to burrow beneath the layer of protective mucus that lies between the digestive juices and the epithelial cell lining of the stomach; once there, it is protected in part by locally secreted bicarbonate. The problem for *H. pylori* is how to penetrate the mucus, which has the remarkable property of transitioning from a viscous solution at neutral pH to a gel-like substance at low pH.

Celli *et al.* demonstrate that the capacity of this bacterium to neutralize its immediate surroundings, via urease activity, also contributes to reducing the viscoelastic moduli of gastric mucin. Measurements of the time-dependent rotation of the bacterial cell body and its flagellum, when trapped within mucin in its gel-like state, reveal that it possesses a high-torque motor operating at roughly 1×10^{-17} N m, a value that is 3- to 10-fold larger than what has been calculated for other bacteria. Adding urea raises the pH, which reduces the moduli by two orders of magnitude and allows *H. pylori* to swim through the mucus at 30 $\mu\text{m/s}$. — GJC

Proc. Natl. Acad. Sci. U.S.A. 106, 10.1073/pnas.0903438106 (2009).

CREDITS (TOP TO BOTTOM): KYLE ELLIOTT; NASA

PLANT SCIENCE

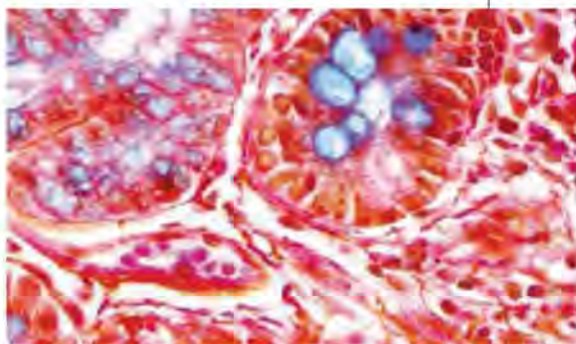
Sending Out an ROS

Local stresses, such as the damage caused by an insect on a leaf, can produce signals that are transmitted systemically to parts of the plant that are not directly injured or stressed. These signals help the plant acclimate to environmental stress or defend against pathogens. Miller *et al.* show that the gene *RbohD*, which encodes a plant NADPH oxidase that generates reactive oxygen species (ROS), is critical for rapid systemic signaling in response to wounding by a scalpel, heating to 42°C, cooling in ice water, high-intensity light, or increased salinity. — NRG
Sci. Signal. **2**, ra45 (2009).

BIOMEDICINE

Deadly Collaboration

By some estimates, more than 50% of the world's population is infected by the bacterium *Helicobacter pylori*, a gastrointestinal pathogen most famous for its role in the development of gastric ulcers. *H. pylori* infection is also a risk factor for gastric cancer, but because only a small percentage of infected individuals develop



Goblet cells (blue) in gastric mucosa.

the disease, it would be of great interest to identify controllable lifestyle factors that might contribute to an enhanced risk of cancer. Correlative data from epidemiological studies have suggested a potential interaction between *H. pylori* infection and diet, but long-term human studies that would establish a cause-effect relationship are not feasible.

In a carefully controlled 5-year study of Rhesus monkeys that were monitored at frequent intervals by gastroscopy and biopsy, Liu *et al.* found that gastric neoplasia (precancerous and cancerous lesions) developed in *H. pylori*-infected animals that had also consumed a carcinogen similar to one found in pickled vegetables and smoked meats, but not in animals exposed to either the bacterium or the carcinogen alone. In terms of cancer prevention strategies, these findings underscore the importance of dietary

awareness in individuals known to be infected with *H. pylori*. — PAK

Gastroenterology **137**, 10.1053/j.gastro.2009.07.041 (2009).

APPLIED PHYSICS

Directing Optical Traffic

Optical applications such as quantum cryptography and communication are reliant on the ability to direct single photons between sender and receiver stations. Though there are several implementations capable of generating single photons, the direction of photon emission tends to be random, and the coupling efficiency into the carrier medium (an optical fiber) tends to be rather low. Toishi *et al.* place a quantum dot in a specially prepared photonic crystal cavity which controls the lifetime of the quantum dot and the direction of the emitted photons. Through careful design of the photonic crystal to match the output modes of the source with the mode of the carrier fiber, they show that they can increase the coupling efficiency into the fiber. The designer optical components should find direct application in opto-electronic circuitry. — ISO

Opt. Express **17**, 14618 (2009).

CHEMISTRY

Edging In with Iron

Hydroboration is widely used in organic chemistry to append a borane and a hydrogen atom to respective carbons at either end of a double bond; the borane group can then be efficiently replaced to form a range of compounds such as alcohols. Wu *et al.* now show that an iron catalyst facilitates a variation on this reaction in 1,3-dienes: hydrocarbon

derivatives with two double bonds separated by a single bond ($C=C-C=C$). Instead of adding boron and hydrogen to adjacent carbons, the reaction proceeds to add them at opposite edges of the four-carbon sequence, concomitantly creating a double bond between the central carbons. The catalyst forms in situ through reduction of an iminopyridine iron(II) complex by magnesium. In substrates substituted at one of the central carbons in the diene framework, the reaction is highly selective for the *E* product geometry about the newly formed double bond, complementing a previously developed palladium-catalyzed variant of this reaction class that favors the opposite stereochemistry. Varying the imine substituent on the ligand also allows the authors to modulate which end carbon receives the boron. — JSY

J. Am. Chem. Soc. **131**, 10.1021/ja9048493 (2009).

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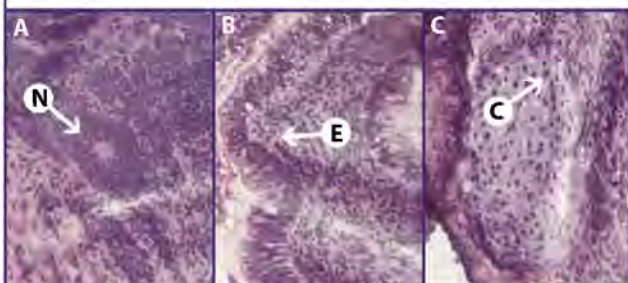
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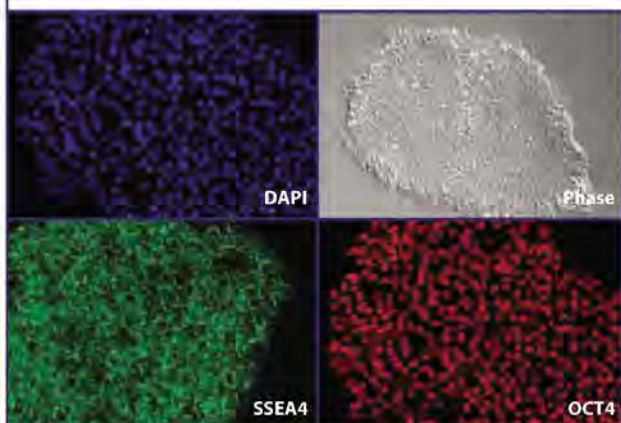
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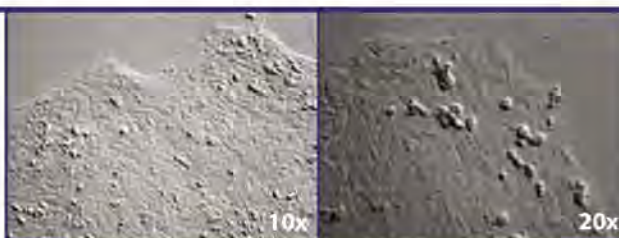
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Teratoma Formation hESCs (H1) cultured with NutriStemTM for 14 passages were injected subcutaneously into SCID mice. Teratomas were harvested after 8 weeks and processed for histological analysis. Tissue types from all three germ layers could be identified in the sections (A-C). (A) ectoderm with neural rosette (arrow marked N); (B) endoderm with columnar epithelium (arrow marked E); (C) mesoderm with cartilage (arrow marked C).



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STARPOPS

Instead of sports heroes or toys, children in Central America have a chance to learn a little about astronomy in the mornings as they munch on their sugar-coated grain products. CIENTEC, a Costa Rica-based nonprofit that promotes public interest in science, has teamed up with the food company Alimentos Jack's to put a series of games and vignettes on the back of its cereal boxes, including the history of space exploration and "stellar bingo."



Divining by Genes

Chinese parents traditionally divined a child's future by putting different objects in front of a 1-year-old baby and watching which one the child reached for. Modern Chinese have found a more scientific-seeming approach. The Chongqing Children's Palace, a school in China's Sichuan region, says it can help children identify their natural talents—and get an edge on their peers—through a simple genetic test.



Cheek swabs are sent to the Shanghai Biochip Corp., which analyzes 11 different genes from each child for clues to the child's emotional and physical potential. The genes include *DRD4*, a dopamine-related gene that research has linked to traits such as impulsiveness and novelty seeking, and *5-HTT*, which helps regulate serotonin and may be related to anxiety and emotional vulnerability. "All genes have a purpose," says project leader Huang Xinhua, director of health studies at Shanghai Biochip.

After testing, the children attend a week-long summer camp during which their per-

formance in activities such as art and sports is analyzed by psychologists and cross-referenced with their genetic data. Eager parents then get recommendations for the children's career paths. Not everyone is impressed. The tests are "not likely to have much basis in reality," says geneticist Richard Myers, director of the HudsonAlpha Institute for Biotechnology in Huntsville, Alabama, adding that they could be "more harmful than helpful."

Roman Horse Found in Germany

Archaeologists in Germany have found a life-sized, bronze, Roman horse's head at the bottom of a well. It's the first such find in Germany and may be one of the world's best-preserved Roman bronzes, scientists say.

The sculpture, part of a statue believed to be of the Roman emperor Augustus, was found at Waldgirmes, the remains of a Roman town. The cast-bronze head, 50 centimeters long, is covered in gold leaf and weighs 25 kilograms.

At a press conference in Frankfurt last week, Friedrich Lüh of the German Archaeological Institute said archaeologists found the horse's head while excavating an 11-meter-deep, wood-reinforced well shaft. Tree-ring dating placed the well's construction at about 9 B.C.E.

It appears that the Romans had grand plans for Waldgirmes, which may have been intended as the capital of what they called *Germania Magna*. There are fragments from five statues in the town, and Lüh says the sophisticated craftsmanship of the horse head indicates that it was made in Italy.

Excavations at Waldgirmes, which started in 1993, are convincing archaeologists that the Romans had a more extensive presence in ancient Germany than historians had thought. "The Romans must have felt so safe, they planted a new town in this wild Germanic forest," says Sebastian Sommer, chief archaeologist of Bavaria.

That safety was short-lived. In 9 C.E., German tribesmen vanquished the Roman legions. Lüh believes Waldgirmes was abandoned at about the same time.





Last-ditch effort
to save the saola

1192



The fraud
that wasn't?

1194

MEDICINE

Disrupting Hedgehog May Reverse Advanced Cancer, If Only Temporarily

In the first clinical proof of its kind, a drug has dramatically shrunk cancerous tumors by disrupting a key genetic pathway. But a study targeting one deadly brain cancer, medulloblastoma, ended in disappointment as the patient's once-tamed tumor quickly developed resistance to the drug and killed him.

The drug, GDC-0449, was developed at Genentech in San Francisco, California. It locks onto and deactivates a protein, Smoothened, that activates the Hedgehog signaling pathway, which in turn orchestrates

26-year-old man with aggressive medulloblastoma, reported in a second paper in *NEJM*. Hedgehog signals have been implicated in one-third of human medulloblastoma cases. Tumors had colonized the man's entire body. "He came in very sick, thinned, in a lot of pain, not very active, needing frequent blood transfusions," says Charles Rudin, associate director of clinical research at Johns Hopkins University in Baltimore, Maryland, who treated the patient. "His prognosis was terrible."

Other treatment options, including radia-

GDC-0449 seems promising for skin cancer, say several experts, and Genentech has already moved into phase II clinical trials. It remains unclear how well the drug will work for medulloblastoma, however. This brain cancer usually targets children, and because the Hedgehog pathway controls aspects of skeletal development, shutting it off might not be safe.

In a separate study published online 2 September in *Science* (www.sciencemag.org/cgi/content/abstract/1179386), a team at Genentech led by biologist Frederic de Sauvage described the mechanism by which the man's brain tumor developed resistance. The team found that a point mutation in Smoothened, a G-to-C substitution at position 1697, prevented GDC-0449 from binding but did not alter the ability of Smoothened to switch on the Hedgehog pathway.

Further research showed that a similar resistance had developed in mice with medulloblastoma. The point substitution was different but occurred in the exact same spot and interfered with the drug in the same way. That correspondence between humans and mice hints that preventing drugs like GDC-0449 from locking onto Smoothened could be a common way to develop resistance. However, scientists can now study this mechanism, and they hope to devise ways around it. Resistance could develop in skin cancers but is less likely because they are rarely treated with mutation-inducing radiation.

Some scientists question the Genentech team's decision to report the medulloblastoma findings in two separate papers in two different journals—especially because many of the authors overlap. Tom Curran of Children's Hospital of Philadelphia in Pennsylvania praised the papers. But he added: "It really should have been one paper. The molecular biology was built on the back of the clinical observation. Having just a single patient case study [as in the *NEJM* paper], it's hard to read much into that." De Sauvage said the work was done separately and that the papers' appearance at the same time was a coincidence.

The class of compounds that includes GDC-0449 could also help contain other cancers, such as ovarian and colorectal cancers, that are not triggered in the same way as medulloblastoma or basal cell carcinoma but that are still partly driven by aberrant Hedgehog signaling. Genentech is already testing drugs along these lines.

—SAM KEAN



Dramatic but transient. Within 2 months, a novel drug candidate shriveled a man's metastasized cancer (center). One month later, the cancer, now resistant, resurged.

how embryonic stem cells develop. In adults, the Hedgehog pathway is dormant, but it awakens in many cancers.

In a paper published 2 September in *The New England Journal of Medicine* (*NEJM*), scientists treated 33 people with one such cancer, basal cell carcinoma, a common skin cancer. The carcinoma had advanced so far that conventional treatments were useless. Nevertheless, after a median treatment of 10 months, 18 patients improved substantially on the drug, and it arrested the spread of cancer in 11 others. Scientists had long suspected that disrupting the Smoothened-Hedgehog link would shrink tumors without messy radiation or chemotherapy. Now they have proof.

Still more dramatic was the response of a

tion, had failed, so in late April 2008, Rudin began giving the man 540 mg of GDC-0449 daily. By 23 June, he had gained 7 kilograms and reported that he was in far less pain than before. He even began exercising again. The numerous blotches on his positron emission tomography (PET) scans had been reduced to a few isolated islands of cancer. But on 23 July, a PET scan revealed that the cancer had returned in a resistant form and was almost as widespread as before. The young man died 23 September.

Despite the ultimate failure of the treatment regimen, Rudin said that "a dramatic reduction, even if transient, seemed worthy of reporting" because no effective treatments for medulloblastoma exist.



The evolution
of cooperation

1196



A broad-spectrum
antiviral?

1200

ARCHAEOLOGY

Ancient DNA Says Europe's First Farmers Came From Afar

About 11,000 years ago, farming began to replace the hunting-and-gathering lifestyle in the Near East. At first, agriculture spread slowly into Europe via modern-day Turkey, Greece, and Bulgaria. But about 7500 years ago, farming suddenly took off in central Europe, spreading in just a handful of centuries from an epicenter in Hungary and Slovakia to as far east as Ukraine and as far west as France. Rectangular houses sprang up, surrounded by cow pastures and fields of wheat and barley. Researchers have long debated whether this agricultural explosion was sparked by massive migrations of farmers themselves, so-called demic diffusion, or by the spread of farming ideas, known as cultural diffusion.

In a paper published online this week in *Science* (www.sciencemag.org/cgi/content/abstract/1176869), European researchers present important new data on this question, for the first time directly comparing ancient DNA from European hunter-gatherers and early farmers. They conclude that outside colonizers brought farming to central Europe in "a major migration event that I never would have believed in before," says team co-leader Joachim Burger of Johannes Gutenberg University, Mainz, in Germany.

Others are impressed but cautious. "It is an important paper" with "very convincing results," says archaeologist Ron Pinhasi of University College Cork in Ireland, who last week reported similar conclusions from a study of skulls from the two groups. Martin Richards, an archaeogeneticist at the University of Leeds in the United Kingdom, says, "I couldn't help but become quite excited," despite his own earlier work supporting cultural diffusion. But Richards and others warn that the findings might be due in part to contamination, long the Achilles' heel of ancient DNA studies.

In the study, researchers led by Burger's Johannes Gutenberg University colleague Barbara Bramanti build on a paper they published 4 years ago in *Science* (11 November 2005, p. 1016). That paper found differences between ancient mitochondrial DNA from early farmers and mtDNA from living Euro-



Not a farming family. Ancient DNA from these 8700-year-old hunter-gatherer skulls from Hohlenstein-Stadel in Germany indicates that they were not the ancestors of later farmers.

peans and was interpreted by many as supporting cultural diffusion.

The new study goes much further by fully sequencing ancient mtDNA from the skeletons of 25 early farmers as well as from 20 hunter-gatherers, thus allowing for direct comparison of the two ancient groups. The bones were previously unearthed at sites in Lithuania, Poland, Russia, and Germany and are dated from about 15,000 years ago to 4300 years ago.

The team found that the mtDNA sequences of the farmers were so genetically distinct from those of the hunter-gatherers that they could not be related. For example, hunter-gatherer skeletons featured a high incidence of two genetic markers, called U4 and U5, which were not found in the farmers, and farmers harbored markers N1a and H, which were not found in the hunter-gatherers. Thus, the first farmers were immigrants who did not immediately mate with the locals.

The team also compared the ancient mtDNA from these two groups with mtDNA from 484 living Europeans. There was little genetic similarity between the hunter-gatherers and modern Europeans, suggesting that most living Europeans descend from the incoming farmers, not the indigenous population. Yet the team also found that the genetic differences between the early farmers and living people were greater than would be expected from genetic drift alone, suggesting

that intervening events—such as additional waves of migration and later admixture with lingering hunter-gatherers—have also shaped the modern European gene pool.

If so, Pinhasi says, the new findings caution against using modern people's DNA to draw conclusions about ancient populations, an approach that has only recently begun to be supplemented by ancient DNA studies. "Much of the genetic signature of early farmers has perhaps been wiped out by subsequent [migrations]," he says.

But Richards points out that the sample sizes of hunter-gatherers—although impressive for ancient DNA—are too small to make sweeping conclusions. If the hunter-gatherers varied regionally, direct comparisons with the farmers might be less reliable. Ancient DNA expert Eva-Maria Geigl of the Jacques Monod Institute in Paris adds that the team's results were not replicated in an independent lab, so contamination cannot be entirely ruled out. "This is a major problem," she says. Bramanti counters that "it is impossible to completely exclude human contamination" but adds that the team was "very scrupulous" in trying to minimize it.

The next step, the researchers say, is to find where those immigrant farmers came from. Burger and Pinhasi are already looking for freshly dug skeletons in western Turkey and southeastern Europe.

—MICHAEL BALTER

VENEZUELA

Dismissal of Senior Scientist for 'Nonattendance' Shakes Community

CARACAS—The dismissal of a top Venezuelan scientist from a government research institute and threats to dismantle one of its most successful programs have revived accusations that the government of President Hugo Chávez is engaging in political persecution. Reinaldo Di Polo, a physiologist with more than 40 years' experience and over 4000 bibliographic citations, learned at the end of July that he was to be removed from his post at the Venezuelan Institute for Scientific Research (IVIC), the country's premier science agency, on the grounds of "nonattendance" on unspecified dates between January and June of this year.

In statements to the press, IVIC Director Ángel Vilorio described the matter as "purely administrative" and accused the press of politicizing it. "It's not a dismissal," Vilorio said. "The man retired on 1 July 1997. He spent all the budget and didn't show up for work. We don't know if all those scientific

articles he says he published were done elsewhere." (Vilorio did not return telephone calls from *Science* seeking further comment.)

Di Polo, along with two dozen other IVIC researchers, belonged to a program known as the PLI (*Permanencia en Labores de Investigación*), which allows retired scientists to continue working. PLI was introduced because Venezuelan law allows academics to retire after 25 to 30 years' service, precisely when a research scientist is at his or her most productive. PLI members represent about 20% of IVIC's research staff, but on average, PLI members are responsible for 50% more articles published in peer-reviewed journals than other IVIC staff, according to PLI member Gioconda San-Blas, head of the mycology laboratory.

Since retiring, Di Polo—who won Venezuela's national science prize in 2000 for his work in neurophysiology—has produced 38 research papers in international journals.

More than half of the research, he says, was done at IVIC. "Nowhere in the world," Di Polo says, "do research scientists have to punch a card." Vilorio, however, is not impressed with academic credentials. "We can't give preponderance to indices of citations created by commercial consortia. We'll have to evaluate how much sense it makes, for the future of the country, to continue counting publications and prizes," he said to the press.

In May, President Chávez called on science minister Jesse Chacón, a retired army lieutenant, to "turn the screws" on unproductive scientists and demanded that research projects have direct, practical applications (*Science*, 29 May, p. 1126). Vilorio, whose own research specialty is butterfly taxonomy, said, "We can't keep giving investment priority to issues that are of no interest to the state." PLI is under review, and "it is likely that this special regime will disappear."

When Chacón visited IVIC on 25 August, he was handed a letter from its Association of Researchers (AsoInVIC), pointing out that, far from being an ivory tower, IVIC addresses issues vital to the country's development. "Just to cite a few examples," the association says, "in the IVIC, research is carried out into many diseases, including

EDUCATION

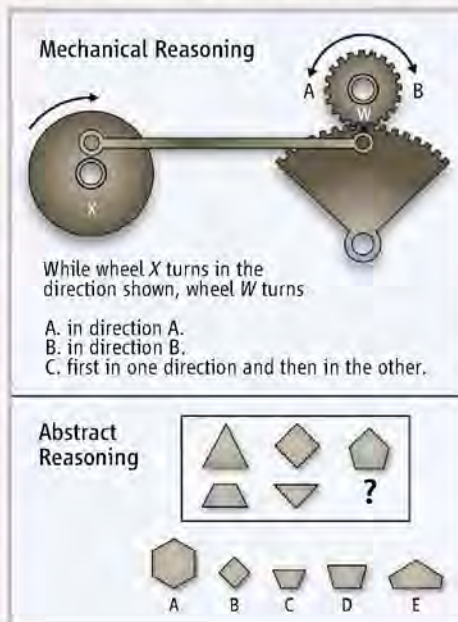
Science Needs Kids With Vision

Albert Einstein, who was famously able to conduct physics experiments in his head, once said his "elements of thought are not words but certain signs and more or less clear images." Einstein would probably make the cut in most modern-day science talent searches. But many of those with exceptional spatial abilities are being missed, claims psychologist David Lubinski of Vanderbilt University in Nashville.

Lubinski, who put his case last week to the National Science Foundation's National Science Board at a workshop on innovation, says that despite their importance in science, particularly in fields such as engineering, robotics, or astronomy, spatial abilities are getting short shrift both in school curricula and in programs trying to spot precocious youths. He estimates that such programs overlook more than half of those with exceptional spatial abilities. "How many Edisons and Fords are we missing?" he asks.

According to educational psychologist David Lohman of the University of Iowa in

Iowa City, spatial ability is "the ability to generate, retain, retrieve, and transform well-structured visual images." Tests cover



areas such as visualization (figuring out what happens when a piece of paper is folded, for example), mentally rotating an object, and mechanical reasoning (see illustration). Many talent hunts for gifted elementary and high school students rely on the results of the SAT, which assess verbal and math—but not spatial—skills.

Lubinski and Camilla Benbow of Vanderbilt have found from their analyses of data from the Study of Mathematically Precocious Youth, begun in 1971 at Johns Hopkins University in Baltimore, Maryland, that there is wide variability in spatial abilities even among the one in 1000 children who score over 700 on the math SAT before age 13. Their latest paper, scheduled for publication in the November issue of the *Journal of Educational Psychology*, shows that spatial abilities "behave in the same way in an average sample" as they do in the hyper-precocious, says Lubinski. Spatial ability roughly correlates with math and verbal ability, but many spatially gifted people are not in the top tier of math or verbal ability. Using data from Project TALENT, a 1960 survey of 400,000 U.S. high school students, they found that among those who scored in

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No slacking. IVIC Director Ángel Viloria (right), with Venezuela's science minister Jesse Chacón (center), ejected one of IVIC's most-published researchers for being out of the office.

dengue, malaria, AIDS, and tuberculosis."

PLI, says Flor Pujol, who is on the board of AsoInIVIC, "is not just good, it is vital, because there is no new generation [to take over]." IVIC researchers are deeply concerned over budget cuts and what they say is a brain drain stimulated by the authorities' dismissive attitude toward them.

Scientists' greatest fear, however, is that the government is intent on eliminating "bour-

geois science" altogether. Claudio Bifano, president of the Venezuelan Academy of Physical, Mathematical, and Natural Sciences, says the country's scientists are "trying to defend internationally accepted principles and values of science and education." The government, he says, "wants to transform this country into Cuba—and that is the real danger."

—PHIL GUNSON

Phil Gunson is a writer in Caracas.

the top 1% of spatial ability, 70% did not make it into the top 1% of math or verbal ability. That means they would have been overlooked by most talent searches. But an 11-year Project TALENT follow-up showed that spatial abilities tend to correlate with scientific achievement. For example, 45% of those with science and engineering Ph.D.s were in the top 4% of the spatial ability range, compared with 25% of the bachelor of science degree holders. Fewer than 10% of the Ph.D.s were below the top quartile in spatial ability.

Such data are news even to people in the field. "I was really amazed at the numbers," says psychologist Nora Newcombe of Temple University in Philadelphia, Pennsylvania, principal investigator for the Spatial Intelligence and Learning Center, a consortium that does research on spatial skills and how to improve them. Newcombe agrees that people with such skills are not getting the attention they deserve in school. As Lubinski says, the typical "highly verbal" high school curriculum "turns them off; they love classes with a big lab component."

The Belin-Blank International Center for Gifted Education and Talent Develop-

ment at the University of Iowa, where Lohman is the research director, is interested in developing and validating a new spatial test to add to those it uses for its talent search and has applied for a foundation grant. A nationally normed test in which results can be reliably compared with math and verbal test scores will offer "a new dimension to help us understand why some are more [scientifically] creative than others," says center director Nicholas Colangelo.

Everyone agrees that spatial tests will disproportionately select boys. Larry Hedges, a statistician at Northwestern University in Evanston, Illinois, has estimated that the ratio of boys to girls in the top 5% of spatial ability is more than 2.3 to 1. For mechanical reasoning, it's about 1.1 to 1. But Hedges also points out that spatial ability is highly malleable: "You can change it much more than IQ or verbal or math abilities."

"It's of concern that you would over-identify males if you just make a decision on the basis of this," notes Newcombe. But, she says, more focus on spatial ability is long overdue: "It's an orphan skill."

—CONSTANCE HOLDEN

ScienceNOW.org

From Science's Online Daily News Site

A Breathalyzer for Cancer

A team of researchers may have come up with a golden idea for diagnosing lung cancer. By coating tiny nuggets of gold with a thin layer of organic material, they've developed an "electronic nose" that, with some additional work, could spot lung cancer instantly by analyzing someone's breath. <http://bit.ly/3xNdJL>

Don't Stand So Close to Me

In a famous episode of the TV show *Seinfeld*, a "close talker" makes others uncomfortable by standing mere centimeters from their faces while speaking. What makes this invasion of our personal space so uncomfortable? A new study fingers the amygdala, a region of the brain that acts like a warning bell when someone gets too close for comfort. <http://bit.ly/3Rr50d>



Glyptodonts Were Savvy Batters

What do ancient armored mammals have in common with Babe Ruth? They both took advantage of the "sweet spot." New research suggests that some species of giant mammals called glyptodonts swung their hefty tails like baseball bats, landing powerful blows with the spot on their tails that minimizes potentially harmful vibrations for the slugger. <http://bit.ly/fiHid>

Global Warming Warps Marine Food Webs

Teasing apart the complex ways in which global warming will affect ocean life has been tough. But new research suggests that a simple ecological theory may explain at least one piece of the puzzle: the effect on marine food webs. And the news may not be all bad. <http://bit.ly/1br735>

Read the full postings, comments, and more on scienownow.sciencemag.org.

ECOLOGY

Last Chance to Save the 'Panda of Indochina'

Is it possible to throw a lifeline to a creature no scientist has ever glimpsed in the wild? "Some days it feels like trying to strategize conservation of unicorns," says William Robichaud, a zoologist based in Laos. But that's exactly the challenge confronting experts who have kicked off an 11th hour effort to prevent the saola, a rare ungulate, from slipping into oblivion.

At an emergency meeting in Vientiane last month, Robichaud and colleagues hammered out a set of measures that, if implemented within the next year, could give the saola a shot at survival. These include intensified removal of poachers' snares and efforts to shed light on the mysterious beast's biology. A central challenge is to elevate saola in the public consciousness—both on the animal's home turf in Laos and Vietnam and in the minds of donors, as funding for saola conservation is almost nil. "We need to sell the saola as the panda of Indochina," says Barney Long, a conservation biologist with the World Wide Fund for Nature (WWF). The animal, which resembles an African desert antelope, "is incredibly charismatic and stunningly beautiful," says Robichaud. "People just don't know it yet."

To wildlife biologists, the saola is the stuff of legend (*Science*, 1 December 2006, p. 1380). It was the first large mammal dis-

Real-world unicorn. An aggressive effort to rein in poachers may be the last, best hope for the saola.



covered in a half-century when researchers described the species (*Pseudoryx nghetinhensis*) in 1992 based on pairs of chestnut-brown, tapering horns hanging in homes in Vietnam's Annamite Mountains. The expansion of settlements and roads have allowed poachers to press deeper into remote Annamite habitat, where they set snares for all manner of wildlife. Villagers have been reporting fewer and fewer saola sightings, suggesting

that the species is fading from the scene. The total population is at most a few hundred individuals—and may be as low as a few dozen, says Robichaud. The few saola kept in captivity all perished within weeks.

With the doomsday clock ticking, the Saola Working Group (www.asianwildcattle.org/awc_s.shtml) of the International Union for Conservation of Nature's Species Survival Commission met to figure out how to

DAM PROJECT REVEALS SECRET SANCTUARY OF VANISHING DEER

When Ulrike Streicher set out last year to rescue wildlife on the Nakai Plateau of northern Laos, nearly half of which was flooding as the reservoir behind the Nam Theun 2 Hydroelectric Project's dam filled, she expected to encounter the occasional curiosity. But in just 4 months, her team captured an astounding 38 large-antlered muntjacs—a rare deer that was discovered only in 1994 and was photographed for the first time

by a camera trap in the dam area in 2007. "We had our hands on more large-antlered muntjacs than anyone had ever even seen," says Streicher.

The hands-on experience could be a boon for efforts to study and protect Indochina's more exotic denizens. Streicher's mostly Lao team attached radio collars to several large-antlered muntjacs (*Muntiacus vuquangensis*) before releasing them in habitat away from the reservoir.

Although the animals aren't presently being monitored—that was beyond the Nam Theun 2 Power Company's mission—"it was a great dress rehearsal for learning how to track animals like the saola," says Streicher, a wildlife veterinarian based in Da Nang, Vietnam, who headed the NTPC wildlife-rescue program.

Jackpot. An amazing 38 large-antlered muntjacs were rescued in Laos.



From June 2008 to February 2009, Streicher's group rescued 294 animals in Nakai's Thousand Island area, including some pygmy lorises and unexpected critters such as the colugo, only the second field record of this gliding mammal in Laos. In a July review, the World Bank commended the rescue program as "impressive." "Uli and her team did a fantastic job, under often difficult conditions," says Laos-based zoologist William Robichaud.

The fate of the large-antlered muntjac—those Streicher released and the population in general—is not rosy. Unlike the saola (see main text), this heaviest of muntjac species—adult males weigh up to 60 kilograms—is a favorite of hunters. "We tried to be secretive about where we brought captive animals," Streicher says. "But when you run into a bunch of locals and you are carrying a couple cages, it doesn't take much imagination to figure out what you're up to." Streicher hopes outside experts will pick up where NTPC left off and join the quest to save the large-antlered muntjac. Otherwise, she warns, "it could be a big scientific loss."

—R.S.

CREDITS (TOP TO BOTTOM): © WWF-CANON/DAVID HULSE; © MARK KOSTICH

prevent the species from sliding quietly into extinction. The group, headed by Robichaud, concluded that saola "cannot be saved" without stepping up the pace of snare removal and curtailing hunting with dogs.

Experts say there are some grounds for optimism. WWF and Vietnam's Forest Protection Department recently launched a snare-removal campaign in newly protected saola habitat in the Thua Thien Hue and Quang Nam provinces. But the limited effort is "far from perfect," says Long, who is hoping a donor will materialize to fund intensive snare removal. In the meantime, he says, "snarers remain a huge problem across Vietnam." In Laos, the Nakai-Nam Theun National Protected Area established an enforcement division this year—"a great ray of hope for the species," says Robichaud. But much of the saola's presumed range in Laos lies outside of national protected areas. How to safeguard those saola, Robichaud says, is "a tough nut to crack."

The working group also called for

improved methods of detecting saola. "The dearth of knowledge of saola ecology, behavior, and current distribution is a significant constraint to planning conservation action," says Robichaud. One approach is to set out more camera traps—an expensive proposition. Another is to train dogs to sniff out saola dung, which could be identified by DNA analysis. But with only one or two certain samples of saola dung on hand from past captives, that's iffy.

The best hope for rescuing one of the world's most critically endangered mammals may be the most direct: Bring poachers to heel. "Unlike so many other endangered species in Asia, the saola has no high price on its head," says Robichaud, primarily because it has no value in traditional medicine and is not an important source of bush meat. "This should make conservation of saola relatively straightforward," he says.

"Donors like winnable causes," Robichaud says. And what could be more enticing than saving a real-life unicorn? —RICHARD STONE

JAPAN

How Will Science Fare?

TOKYO—In an annual rite of summer, the education ministry's budget requests are trimmed by advisory bodies, politicians, and the powerful finance ministry. This year, there is a new twist: The newly elected Democratic Party now has responsibility for finalizing the budget—and no one knows how R&D will fare.

The Democratic Party's platform keys in on the importance of research. But the party also promised to cut wasteful governmental spending, without being specific, and party politicians have called for reining in bureaucracy. "It's very difficult to say at this stage what to expect," says Reiko Kuroda, a biochemist at the University of Tokyo and a former member of the Council for Science and Technology Policy, the nation's top science advisory body.

The budget request, released on 28 August, includes a new \$110 million program to hire graduate students as teaching assistants; a 17% increase, to \$2.4 billion, for grants-in-aid for scientific research that supports individuals and small groups; a 29%



New era. Yukio Hatoyama, the Democratic Party chief, wants to keep tabs on Japan's bureaucracy.

increase, to \$98 million, in funding for regenerative medicine; and a 35% jump, to \$2.8 billion, for the space program. The Democratic Party, which has called for more green-energy schemes, might back the ministry's plan to expand R&D into making Japan a low-carbon society by 75%, to \$506 million.

Hidefumi Kobatake, president of the Tokyo University of Agriculture and Technology, points out that the ousted Liberal Democratic Party squeezed support for national universities by

about 1% in each of the past several years; the Democratic Party has pledged to put a stop to the cuts. "We'll be extremely thankful if that happens," Kobatake says.

The ministry has not yet totaled up science-related spending, and at best the overall science budget will increase "by a few percent," says Shuichi Sakamoto, director for budget planning for the ministry. The budget will likely be finalized by December; it takes effect next April.

—DENNIS NORMILE

ScienceInsider

From the Science Policy Blog



The much-ballyhooed report on the future of the **U.S. human space program** was submitted to the White House on 1 September, or so rumor has it. The so-called Augustine report is the latest in a series of analyses of pressing issues affecting the research community—scientific integrity and biosecurity being the others—that the Obama Administration has chosen to keep under wraps.

In the most comprehensive report yet on **geoengineering**, Britain's Royal Society calls for more research but cautions that the many impacts of global warming won't be solved by any single technology. Many approaches could have substantial side effects, such as worsening drought.

India's moon mission, **Chandrayaan-1**, has come to a shuddering and unexpected halt. The Indian Space Research Organisation lost all contact with the \$100 million spacecraft on 29 August after a catastrophic failure of its electronics.

The U.S. Coast Guard wants feedback on a draft regulation designed to prevent **invasive species** from entering U.S. waters in the ballast water of ships. The Coast Guard says it "will work to elevate the priority" of research to figure out how effective the measure will be.

Citing new test results, Geron Corp. expects to resume its phase I clinical trial using **embryonic stem cells** to treat spinal cord injury. The U.S. Food and Drug Administration halted the trial after some animals developed small cysts. Spinal cord patients routinely develop much larger cysts.

The Department of Veterans Affairs (VA) announced on 27 August it would opt out of a controversial research project on **Gulf War illness**, citing "persistent noncompliance and numerous performance deficiencies." UT Southwestern officials "strongly disagree with the VA's characterization of the facts."

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Acquired inheritance. Instead of carrying eggs on their legs, Kammerer's midwife toads and their offspring started laying eggs in water.

HISTORY OF SCIENCE

The Case of the Midwife Toad: Fraud or Epigenetics?

Paul Kammerer has been called the perpetrator of one of the most celebrated scientific frauds of the early 20th century. He has also been defended as the victim of forgery by a lab assistant; some even say he was framed by political or scientific opponents. Now, a new analysis published this week suggests that his infamous experiments may have been the first demonstrations of a recently recognized phenomenon: epigenetics.

The story starts in the early 1900s. Kammerer, an Austrian biologist, argued strongly in favor of the Lamarckian view that traits acquired during an organism's lifetime could be passed on to future generations. He claimed to have observed Lamarckian inheritance in various organisms, including salamanders and tunicates, but his most publicized evidence came from the midwife toad, *Alytes obstetricans*.

Most frogs and toads mate in water and lay their eggs in aquatic environments. But midwife toads are landlubbers. Males and females copulate on dry land, and males subsequently wrap the strings of eggs around their legs, carrying them around until the embryos are ready to emerge as tadpoles.

Toads that mate underwater have special colored calluses on their forelimbs, called nuptial pads, that enable them to grasp a wet, slippery female. Given their terrestrial preferences, midwife toads lack nuptial pads—at least they did when Kammerer started his experiment.

He confined the toads to a dry, overheated environment, driving them to mate and lay their eggs in water. Most of the eggs died, but

the 3% to 5% of offspring that survived had lost the terrestrial habits of their parents. Even in cool, moist environments, they opted to mate and deposit their eggs in water, a preference that Kammerer said persisted for at least six generations, the amount of time Kammerer studied them.

Moreover, by the third generation, Kammerer reported a thickening on the forelegs that, two generations later, was a bona fide nuptial pad. Other traits useful for an aqueous existence appeared and became more pronounced: Eggs developed thicker jelly coats and reduced quantities of yolk; tadpole gills expanded in size.

Finally, when Kammerer bred the “water” toads with untreated toads, he saw these “water” traits appear in the proportions one would expect for Mendelian inheritance. According to science historian Sander Gliboff of Indiana University, Bloomington, Kammerer thought new genes were forming to pass on these traits.

Some of Kammerer's colleagues were aghast, and he came under attack from giants of the scientific establishment. The final blow came in 1926 when herpetologist G. Kingsley Noble of the American Museum of Natural History in New York City examined the last remaining specimen of Kammerer's “water” toads and noticed that India ink had been

injected into the toad's forelimbs. “We have proved conclusively that no pads are present,” Noble wrote in the 7 August 1926 issue of *Nature*. “Whether or not the specimen ever possessed them is a matter for conjecture.” Noble and his colleagues also discounted Kammerer's photos of pads and stained sections, arguing that they could not be verified as belonging to midwife toads.

Less than 2 months later, Kammerer killed himself in the Austrian mountains. Some say he committed suicide because he was discredited; others blame unrequited love. Whatever the cause, Kammerer continued to spark controversy long after he was gone. A book about the case by Arthur Koestler, a TV documentary, and a Soviet movie all portrayed him as a victim. Kammerer is “either cited as one of the paradigms of scientific fraud, how a brilliant person can go astray, or by others as a hero for any form of antiestablishment cases in the sciences,” says Günter Wagner of Yale University.

Now comes Alexander Vargas, an evolutionary developmental biologist at the University of Chile in Santiago, who has taken a close look at the case from a 21st century perspective. In the *Journal of Experimental Zoology Part B: Molecular and Developmental Evolution*, released on 3 September, he argues that Kammerer's experiments show signs of what are now well-known epigenetic effects. “There are potential mechanisms that can explain these observations that weren't

available at that time,” says Azim Surani, a developmental biologist at the University of Cambridge in the United Kingdom. Adds Vargas: It suggests “a tragic case of scientific incomprehension.”

Acquired traits can seem to be inherited because they result from chemical modification of DNA that can be passed on—even enhanced—in subsequent generations. The sequence stays the same, but the addition or removal of methyl groups silences certain genes. These changes are the guts of epigenetics.

Vargas points out that Kammerer also noticed a “parent of origin” effect in which a trait tends to appear only if it's passed on by the right parent. In Kammerer's experiments, if the father was a “water” toad, then 100% of the first generation and three-quarters of the next generation were “water” toads as well. But if the father retained the toad's terrestrial habits, then the reverse was true. This odd observation “only complicated the scenario for Kammerer, increasing suspi-



Victim of his time? Austrian biologist Paul Kammerer was discredited for work that may not have been wrong after all.

CREDITS (TOP TO BOTTOM): NEIL HARDWICK/PHOTOGRAPHY, PHOTOGRAPHEDIRECT.COM, © BETTMANN/CORBIS

cions about his data," Vargas writes. Similar effects have been noted in the inheritance of coat colors in mice, which have been ascribed to epigenetic changes in a gene variant called *Agouti variable yellow*, Vargas notes.

Vargas suggests that alterations in the jelly coat around the eggs of Kammerer's toads could lead to abnormal methylation of some genes. All vertebrates have such a coating, and its removal just after fertilization can lead to a reduction in DNA methylation in very early embryos, Vargas points out.

Altered methylation patterns affect body size in mammals, particularly hybrids, and parent-of-origin effects influence egg size in

birds and may likewise have led to the large water toads and small eggs. "Kammerer could be the true discoverer of non-Mendelian, epigenetic inheritance," Vargas concludes. And the intensification of these traits over subsequent generations could be a reflection of ever-increased amounts of methylation—akin to the increased darkening of mice carrying a more heavily methylated *Agouti variable yellow* gene.

Vargas suspects that epigenetics brought out the water traits and that the nuptial pads were a rare recessive trait connected with traits that enabled that small percentage of midwife toads to survive a water gestation. Eventually, two survivors, each with a recessive gene for

nuptial pads, mated, causing the pads to appear.

Vargas doesn't explain the India ink that ultimately led to Kammerer's undoing. Koestler and others have ascribed it to an overzealous lab assistant or even Kammerer's scientific or political opponents.

Gliboff is not completely swayed by Vargas's arguments. "It's still hard to see Kammerer as anything but a failure as a scientist," he says. But Surani's curiosity is piqued. "It would be extremely interesting if someone did really try to repeat [Kammerer's] experiment," he notes. "I wouldn't be surprised if he turned out to be right."

—ELIZABETH PENNISI

HIV/AIDS RESEARCH

Potent HIV Antibodies Spark Vaccine Hopes

If HIV/AIDS researchers had a wish list, at the very top would sit a vaccine that could teach the body to make potent antibodies against the many strains of the virus. Despite 25 years of effort, no such vaccine is in sight, but now they are a step closer. A large team of researchers has identified the most powerful, broad-acting antibodies yet against multiple strains of the virus.

Finding good antibodies is a far cry from developing a vaccine that prods the immune system to produce them. But "broadly neutralizing antibodies" (bNAbs) are rare: Researchers have identified only a half-dozen to date. Now an international group funded mainly by the International AIDS Vaccine Initiative (IAVI) has discovered two new ones that have an unusual potency. "This has actually made me quite optimistic—for once," says Dennis Burton, an immunologist at the Scripps Research Institute in San Diego, California, who led the research effort.

For many years, Burton says, he thought that if an antibody had a broader reach, it inevitably would be weaker. "I wondered whether there would be any antibody better than the ones we had," he says. "Well, these are."

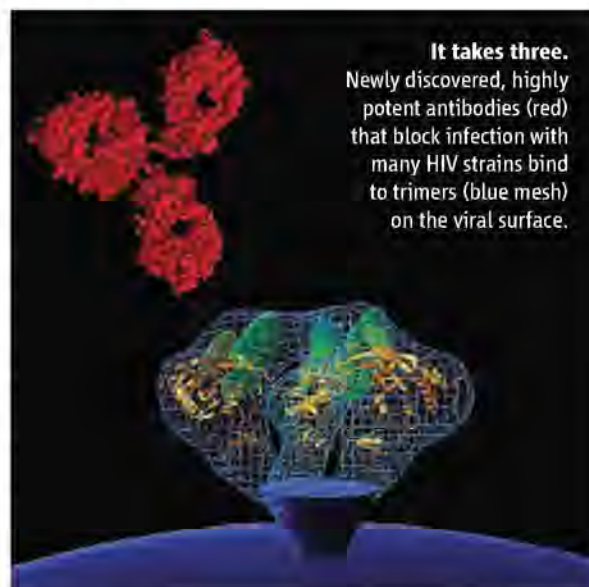
Burton, his graduate student Laura Walker, and 33 other researchers report online 3 September in *Science* (www.sciencemag.org/cgi/content/abstract/1178746) that the two new antibodies have unusual characteristics that open new avenues of AIDS vaccine research. "It's a great paper that describes very novel antibodies," says immunologist John Mascola of the Vaccine Research Center at the National Institute of Allergy and Infectious Diseases in Bethesda, Maryland.

The researchers first collected blood from some 1800 HIV-infected people in Africa, Asia, Europe, and North America. Using novel techniques, they identified 10% who had antibodies that could derail more than a dozen different strains of the virus. This paper focuses on one sub-Saharan African donor; the person did not benefit appreciably from the antibodies, which are no match for HIV once an infection is established.

The researchers sifted through a staggering 30,000 antibody-producing B cells from the donor and isolated two monoclonal antibodies, dubbed PG9 and PG16, that could prevent infection in more than 70% of 162 viral strains tested in cell culture. Not only were they broad acting, but the antibodies worked at minute levels—a magnitude lower than the four best characterized bNAbs so far. "It's an enormous amount of work—a tour de force," says AIDS vaccine researcher Ronald Desrosiers, head of the New England Primate Research Center in Southborough, Massachusetts.

On a more sobering note, many researchers have tried to make vaccines that elicit previously identified bNAbs. "In the last 5 years, there have been intensive efforts, and no one has succeeded," Burton says.

Still, Burton and others hope that understanding the unusual way that PG9 and PG16 stop the virus will provide new leads for AIDS vaccine designers. Specifically, HIV's surface proteins attach to immune cells to establish infections. The surface proteins nat-



It takes three.
Newly discovered, highly potent antibodies (red) that block infection with many HIV strains bind to trimers (blue mesh) on the viral surface.

urally occur in clusters of three, or trimers, and PG9 and PG16 work only against the trimer. Other bNAbs bind to trimers as well as single surface proteins, or monomers. So this suggests that if a vaccine can present the surface proteins to the immune system in the trimeric form, it may have extra punch. It might also help explain why several AIDS vaccines that contain monomeric surface proteins have performed poorly.

Wayne Koff, who heads research and development at IAVI, says PG9 and PG16 are the first of several new bNAbs that he predicts will help guide the field. In particular, researchers hope the antibodies might help crystallographers finally elucidate the structure of a trimer, which occupies another slot on the wish list. "The machine is built and ready to crank out a lot more—and it's very likely to," says Koff.

—JON COHEN

On the Origin of Cooperation



COOPERATION HAS CREATED A CONUNDRUM for generations of evolutionary scientists. If natural selection among individuals favors the survival of the fittest, why would one individual help another at a cost to itself? Charles Darwin himself noted the difficulty of explaining why a worker bee would labor for the good of the colony, because its efforts do not lead to its own reproduction. The social insects are “one special difficulty, which first appeared to me insuperable, and actually fatal to my theory,” he wrote in *On the Origin of Species*.

And yet cooperation and sacrifice are rampant in nature. Humans working together have transformed the planet to meet the needs of billions of people. Countless examples of cooperation exist between species: Cleaner fish pick parasites off larger fish, and nitrogen-fixing bacteria team up with plants, to name just a few.

In some cases, cooperation has fueled key evolutionary transitions, helping to create integrated systems. Worker ants have no offspring of their own and instead feed their queen's offspring in colonies often considered “super-organisms” many thousands of individuals strong. Cells managed to specialize and stay together, giving rise to multicellular organisms. “At each of those levels, formerly independent reproductive units and targets of selection become integrated into a single reproductive unit and target of selection,” notes biologist James Hunt of North Carolina

State University (NCSU) in Raleigh.

So pervasive is cooperation that Martin Nowak of Harvard University ranks it as the third pillar of evolution, alongside of mutation and natural selection. “Natural selection and mutation describe how things change at the same level of organization,” he explains. “But natural selection and mutation alone wouldn't explain how you get from the world of bacteria 3 billion years ago to what you have now.” Cooperation leads to integration, and integration to the complexity we see in modern life.

The challenge of cooperation is to explain how self-interest is overcome given the way natural selection works. Darwin suggested that selection might favor families whose members were cooperative, and researchers today agree that kinship helps explain cooperation. But cheaters—those who benefit without making sacrifices—are likely to evolve because they will have an edge over individuals who spend energy on helping others, thus threatening the stability of any cooperative venture. That puzzle has inspired biologists, mathematicians, even economists to come up with ways to explain how cooperation can arise and thrive. Researchers have spent countless hours observing social organisms from man to microbes, finding that even single-celled organisms have sophisticated means of working together. As genomics has come of age, researchers are getting down to the genetic nuts and bolts of cooperation in a variety of systems for the first time.

All in the family

To help explain the puzzle of how cooperation evolved, Darwin suggested that it might benefit members of a family to help each other. The British biologist William Hamilton took this idea to heart in the 1960s. He formalized modern thinking about cooperation with the proposal that the offspring of relatives counted toward one's individual fitness. Relatives' progeny share some of your genes, so helping them furthers the spread of the shared genes. The more offspring relatives produce, the more those

genes will spread. Helpers will therefore evolve if their overall reproductive output rises enough to make up for any loss of direct reproductive output.

Hamilton worked out a formula for predicting “inclusive fitness”—your offspring plus some proportion of your relatives' offspring—and used that as a guide to determining whether cooperation should evolve in various circumstances. He concluded that inclusive fitness could indeed explain the evolution of highly social insects, whose colonies are composed of related individuals, thus solving Darwin's problem.

This concept implies that individuals will behave differently depending on the degree of relatedness. As another Brit, J. B. S. Haldane, was reported to have put it 30 years earlier, “Would I lay down my life to save my brother? No, but I would to save two brothers or eight cousins.”

The idea that kinship helps drive cooperative behavior has proved a powerful one, but it cannot explain all of cooperation. Humans, for example, often cooperate with nonrelatives. “No other species seems to have succeeded in establishing large-scale cooperation among genetically unrelated strangers,” wrote economist Ernst Fehr of the University of Zurich, Switzerland, in the 25 November 2004 issue of *Nature*.

To help explain our cooperative nature, in the 1970s, Robert Trivers, now at Rutgers University in New Brunswick, New Jersey, came up with the idea of reciprocal altruism—“You scratch my back, and I'll scratch yours,” as he put it. Researchers inspired by his work used computer simulations to approximate what might happen in real life over many generations. Programmers created games in which two players had the option to cooperate and

found that cooperation could evolve and be maintained between two people if they did “tit for tat,” following each other's lead in deciding whether to cooperate.

But this progress can't explain how large cooperative groups—in which the chances of encountering a helper or a helped person were small—could evolve. Also, researchers had to consider the prospect of cheating: When multiple individuals work together to find food, make a home, or defend their communities, if a few people fail to contribute and became freeloaders, others may follow, destabilizing cooperation altogether.

THE YEAR OF DARWIN



This essay is the ninth in a monthly series. For more on evolutionary topics online, see the Origins blog at blogs.sciencemag.org/origins. For more on cooperation, listen to a podcast by author Elizabeth Pennisi at www.sciencemag.org/multimedia/podcast.



In 1998, Nowak and Karl Sigmund of the University of Vienna proposed a way around these issues, at least in the case of humans. They developed a mathematical model suggesting that people decide what to do based not only on whether others have helped them but also on whether others have helped others. “Reputations were also important,” Nowak explains. A person with a reputation for helping gets help, even from someone who has not benefited directly from that person in the past. This could allow cooperative strategies in games to be successful and, by extension, cooperative societies to evolve, Nowak argued.

Other game-playing experiments bore this out. In 2004, Robert Boyd of the University of California, Los Angeles, showed through simulations that this strategy worked particularly well if those who did not help or had a reputation for being slackers were shunned and were refused help.

But Fehr thought even this didn’t fully explain humans’ extremely cooperative nature. In his labor market studies, he found that people tend to be more cooperative than economic theory would predict: Fairly paid employees voluntarily worked harder than predicted based solely on self-interest, for example. He also noticed that people tended to cooperate with strangers, even though there is little chance these interactions would affect their reputations. He wondered what motivational and social forces might drive and sustain this behavior.

After working with many game-playing experiments, Fehr suggested that punishment also plays a key role in making cooperation successful. In 2002, he reported an experiment in which participants decided whether to keep money they were given or contribute some or all of it to a group project. Participants also had the option to punish non-contributors. Punishment was rampant, and about 75% of the time, it was the above-average contributors—“cooperators”—who penalized freeloaders. When punishment was not part of the game, average contributions

“The same core theories that are used to understand cooperation in humans and other animals can also be applied to microbes.”

—Kevin Foster,
Harvard University

dropped. Other work has pointed out that over the long term, the mere threat of punishment—rather than punishment itself—is enough to inhibit cheating. So the cost to punish decreases, but the benefit remains.

Nowak thinks altruistic leanings may in part be instinctive, having evolved because for most of human history, small, related groups were the norm, and reputations were always at stake. But he downplays the importance of punishment, as it may have long-term negative effects such as escalating interpersonal conflicts. When individuals encounter each

One for all. Cooperation comes in many forms, among species—as with cleaner shrimp and fish tending a moray eel (*far left*), as well as within species, as in (*left to right*) ants, lions, and wasps.

other repeatedly, “rewards work much better” in perpetuating cooperation, he insists. In the 1 January issue of *Nature*, he and his colleagues concluded, based on modeling experiments, that punishment rarely pays and that refusing to help noncooperators is much more effective. And on page 1272, they report that in repeated games involving 192 subjects interacting in groups of four via a computer, reward led to increased contributions from individuals and a greater payoff for the group. Punishment can work to keep cooperation going, he says, but in those experiments, punitive actions led to a lower payoff and did not change how much people contributed to the group. Given that Fehr’s experiments show the opposite, the role of punishment is still a matter of debate.

Group selection

Others have applied a different focus when thinking about the evolution of cooperation. They have become convinced that competition among groups can foster cooperation



All for one. Studies of hunter-gatherers such as the Hadza help clarify how cooperation arose in humans.

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within them. In other words, evolutionary forces can act on several levels, with natural selection's push to make individuals less cooperative being countered by competition at the level of the group, because groups with greater cooperation among members tend to survive better. Darwin noted in *The Descent of Man* that this seemed to be true of human groups. If two tribes were competing, and "the one tribe included a great number of courageous, sympathetic and faithful members, who were always ready to warn each other of danger, to aid and defend each other, this tribe would succeed better and conquer the other," he wrote.

That is true for more modern warring groups, says Samuel Bowles, an economist at the Santa Fe Institute in New Mexico. "From military history, it's [known] that groups that are more likely to cooperate are more likely to be successful," he says. Frequent violent encounters with other human groups made such cooperation essential in our past, Bowles and his colleagues have argued.

rates in 20th century Europe with its two world wars. Such frequent warfare made altruistic cooperation among group members essential to survival, says Bowles.

Using game theory, he has simulated what happens in war and concluded that humans could have easily evolved what he calls parochial altruism—wherein you help others in the group, independent of familial relationship, and harm outsiders. "It could have promoted a predisposition to cooperate in groups even at considerable cost to the actor," says Bowles.

From man to microbe

A lot of effort has gone into understanding how humans got to the point at which they could get along, and a lot of cooperative theory has been developed with *Homo sapiens* in mind. But it doesn't take a large brain and a winning smile to cooperate. Even bacterial viruses called phages have prospered by working together. In 2005, Joel Sachs and James Bull, both then at the University of

better, "the same core theories that are used to understand cooperation in humans and other animals can also be applied to microbes," says Kevin Foster of Harvard. By working with yeast, bacteria, and amoebas, several teams have been able to discern fundamental principles about the evolution of cooperation. "Microbes are experimentally tractable, ... and evolutionary dynamics occur over laboratory time scales," says Jeffrey Gore of the Massachusetts Institute of Technology in Cambridge. Relatedness, cheaters, and other factors all come into play to determine the success of these microscopic cooperative ventures.

For example, Stuart West, an evolutionary biologist at the University of Edinburgh in the United Kingdom, studies a group phenomenon called quorum sensing in the opportunistic pathogen *Pseudomonas aeruginosa*. Like other bacteria, this pathogen secretes chemical signals that fellow *Pseudomonas* bacteria in turn respond to by releasing a variety of products, including virulence factors, nutrient-scavenging molecules, and compounds that become the scaffolding for aggregates of microbial cells called biofilms. In the lab, West and his colleagues helped to demonstrate that when *Pseudomonas* individuals sense the accumulation of other *Pseudomonas* nearby, thanks to the increasing concentration of certain biochemicals, they increase their secretion of these helpful substances, providing a benefit to all *Pseudomonas* present.

Two years ago, West's team also showed that this system, like those of humans playing evolutionary games, was vulnerable to cheaters, bacteria that secreted no helpful substances but reaped the benefits of those that did. In one experiment, after 48 hours and seven generations, the population of one type of cheater increased from 1% to 45% of the colony, raising the question of how the cooperative wild type could possibly persist. West thinks that relatedness may be the answer: Wild-type bacteria tend to cluster in high densities, crowding out the mutant cheaters, which have a different genetic makeup.

In the 24 February issue of *Current Biology*, West's team extended these findings by exploring cooperation and cheating in *Pseudomonas* when it was growing in high densities on burn wounds in mice. The proportion of cheaters affected the well-being of both microbes and mice, they reported. When more cheaters were present, the mice did better—possibly because fewer virulence factors were being produced—suggesting that one might treat infections of quorum-sensing microbes by introducing cheaters into their midst, says West.



Helping hands. Rescuers save a swimmer in this drill in China; such cooperative behavior toward non-relatives is rare outside humans.

Their most recent study backs up that contention. In the 5 June issue of *Science* (p. 1293), Bowles evaluated archaeological evidence from about 50,000 years ago, as well as ethnographic and historical reports on certain hunter-gatherer populations—those whose lifestyles, he argued, came the closest to resembling ancient human societies.

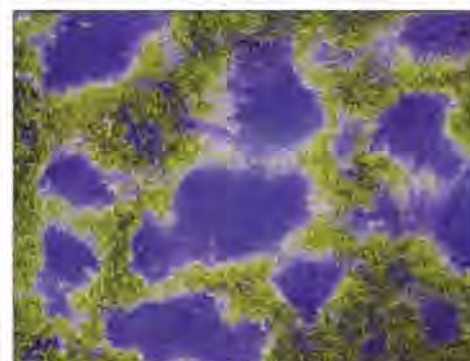
Bowles found that the percent of adult deaths due to warfare ranged from 0% to 46% at the different sites, averaging out to about 14%—significantly higher than the death toll

Texas, Austin, demonstrated that traits that reduce competing self-interest evolved in two different types of phages. The two phages were introduced into a bacterial strain at the same time. Over many generations, the two began to package their genomes within a single protein coat, ensuring that both would be transmitted to the next host bacterium. One phage eventually lost the genes needed to make its own coat, they reported.

The more researchers look, the more cooperation they find among microbes. Even



Cooperating microbes. A composite scanning electron micrograph (above) shows individual slime mold amoebae forming a fruiting body. *Pseudomonas aeruginosa* strains can swarm (top right) to create a biofilm (middle right) to make better use of resources. And in a group of yeast cells exposed to ethanol, the outer cells died (blue), but they protected inner cells from harm.



Perhaps the most celebrated social microbe is the slime mold, *Dictyostelium*, an organism long studied by developmental and cell biologists as a model. More than a century ago, researchers showed that these single-cell amoebae sometimes merge to form stalked fruiting bodies, which produce spores that disperse to more food-rich environments. In the 1980s, researchers recognized that the amoebae forming the stalks were altruistic, giving up their chance at reproduction to help position other amoebae to produce spores. Studies by David Queller and Joan Strassmann of Rice University in Houston, Texas, show that, as with other organisms, cooperation among slime mold amoebae involves tradeoffs and that relatedness matters.

Cheaters are a constant threat: In 2000, Richard Kessin of Columbia University screened for and found such cheating cells, mutants that manage to avoid becoming part of the nonreproductive stalk and instead infiltrate only the fruiting body. If cheaters make up half the population of aggregating amoebae, they can make up about two-thirds the spores in the fruiting body. Working with Baylor College of Medicine and Rice colleagues Gad Shaulsky and Adam Kuspa, Queller and Strassmann have now found more than 100 slime mold genes that confer the ability to cheat. These genes cover the gamut of functions and are involved at differ-

ent points in the development of the fruiting stalk. "The large number of genes and pathways involved suggest that it may be easy to evolve cheating and difficult to control it fully," the authors wrote in the 28 February 2008 issue of *Nature*.

But their work has also shown that amoebae can keep cheating in check, in large part because the mutations that make cheating possible also tend to keep cheaters from getting into the aggregations at all. In laboratory tests, where it's easier to move about, amoebae lacking the cell-adhesion gene called *csaA* tend to bypass the stalk and settle in as part of the fruiting body, acting as cheaters. But on soil in the wild, the lack of this adhesion protein keeps them from getting into the fruiting-body formation in the first place, says Queller.

The *csaA* gene is an example of a so-called green-beard gene, a gene that enables an individual to recognize—as one could recognize a green beard—and cooperate with others who carry that same gene. Those with green-beard genes help perpetuate copies of the gene in others, regardless of the degree of relatedness among individuals. In the case of slime mold amoebae, the *csaA* proteins bind to each other, preferentially linking cells that share this green beard, so that they can produce a stalk and fruiting body.

Few real-world green-beard genes are known. But yeast have one, too—another cell-adhesion protein called FLO1 that leads to clumps, or "flocs," of individual yeast cells, as shown in 2008 by Harvard's Foster, Kevin J. Verstrepen of the Catholic University of Leuven in Belgium, and colleagues. As in the slime mold amoebae, only yeast with the gene can come together. When the yeast are in a floc, outer cells inadvertently become altruistic, as they shield inner cells from toxins and other environmental stresses, often at a cost to their own well-being.

That yeast and slime molds have been shown to possess green-beard genes speaks to the power of microbial systems to help pin down how cooperation works: Hamilton had predicted the existence of such genes long before they were discovered. Countless other organisms, from termites to meerkats, provide additional opportunities for the study of cooperation. "The origin of sociality is unlikely to be encompassed by a single explanation," says NCSU's Hunt. "Sociality, like multicellularity, has appeared numerous times, in diverse taxa, and reached many different levels of integration."

—ELIZABETH PENNISI



Sugary Achilles' Heel Raises Hope For Broad-Acting Antiviral Drugs

For drug developers, viruses make difficult targets. Their ability to co-opt our cells' own machinery makes them hard to attack without inflicting collateral damage, and their outermost proteins are often covered by sugar groups that shield them from human immune surveillance. But now researchers may have found a way to turn viruses' sugary camouflage into a vulnerability.

Over the past several years, researchers at the U.S. National Cancer Institute (NCI) have discovered three antiviral proteins that bind to sugar groups that commonly decorate viral proteins but are less common on human proteins. Once attached, the antiviral proteins can gum up a virus's machinery for entering cells. The most potent of these, known as griffithsin (GRFT), was first isolated in 2005 and proved to be a potent inhibitor of HIV. At the ACS meeting, Barry O'Keefe, a protein chemist with NCI's Molecular Targets Development Program, reported that GRFT does nearly as well against the SARS and Ebola viruses as it does against HIV. GRFT increasingly appears to be a powerful broad-spectrum antiviral.

"It seems to be very promising," says Daron Freedberg, a carbohydrate protein specialist

with the U.S. Food and Drug Administration's Center for Biologics Evaluation and Research in Bethesda, Maryland. So far, GRFT has been tested only in animals and in cell cultures. But if it works in humans without unacceptable side effects, O'Keefe says, its stability and cheapness to manufacture could make it particularly useful in developing countries.

After discovering GRFT and its kin in standard screenings of natural-product extracts from marine organisms, O'Keefe and his colleagues quickly found that all of the proteins had an affinity for binding to a sugar group called mannose when it is at the end of a chain of sugars. Many newly minted human proteins also sport mannose, but the sugar group is usually later modified in an intercellular compartment called the Golgi apparatus. That means viral proteins, not human proteins, are vulnerable to compounds targeting mannose-rich sugar complexes on proteins.

GRFT does that with gusto. Each GRFT binds to three mannose groups, O'Keefe said at the meeting. Because GRFT molecules typically team up to work in pairs, or dimers, each dimer binds to six mannose groups. When GRFT finds a mannose-rich target, the six mannose binding sites lock it up and don't let go.

Natural wonder. A compound found in red algae shows promise against HIV, SARS, and Ebola.

For nearly all HIV strains, it takes less than 0.23 billionths of a mole, or nanomoles, of GRFT to inhibit half the viruses in vitro—a standard measure of drug effectiveness known as the compound's IC_{50} , in which the lower the number the more potent the compound. For SARS, GRFT's IC_{50} is about 50 nanomoles, and for Ebola it is 380 nanomoles. That makes all three mannose binders some of the most powerful antivirals around.

The animal studies are equally compelling. In mice infected with SARS, 70% of the animals that received no antivirals died. By contrast, among those that received an intranasal dose of 5 milligrams per kilogram per day of GRFT for 4 days, 100% lived. With mice exposed to Ebola, one of nature's most lethal viruses, all of the 10 control animals that didn't receive GRFT died within 12 days. In the five groups of 10 animals that each received different injected doses of GRFT, up to 90% survived. Even when they were injected with the antiviral 2 days after being exposed to Ebola, 30% still lived.

O'Keefe says the NCI team plans to test GRFT on flu viruses, including novel H1N1. Meanwhile, one drug company is gearing up for human trials of an anti-HIV formulation, and others may soon be on the way.

New Trick for Splitting Water With Sunlight

For solar power to become our foremost energy source, researchers will need to find a way to store and move it—preferably in the form of chemical fuel. Plants do that through photosynthesis, with the help of a manganese-based catalyst. At the ACS meeting, a team reported incorporating a mimic of this natural catalyst into a solar cell to create what amounts to artificial photosynthesis: a device that turns sunlight into fuel.

"This is a very nice observation. It's a real step forward," says Michael Graetzel, a chemist and solar energy expert at the Swiss Federal Institute of Technology in Lausanne. Gerhard Swiegers, a chemist at the University of Wollongong in Australia and a member of the team that reported the new work at the meeting, cautions that the new cells are still too inefficient to make hydrogen at a commercially viable price. But he adds that the

ALTERED MICROBES MAKE DARK-HORSE BIOFUELS

If researchers can't get the sun to generate fuel directly, some hope microorganisms can finish the job. Two results at the meeting pushed that hope forward. In one, bioengineers equipped photosynthetic algae with the metabolic equipment needed to make isobutanol, a potential alcohol biofuel. In the other, researchers tweaked a reluctant microbe to make large quantities of *n*-butanol, another commercially important chemical.

Most biofuel efforts have focused on using microorganisms to convert the plentiful sugars in corn and sugar cane into ethanol. Ethanol, however, is far from an ideal fuel. It contains only about 70% as much energy by volume as gasoline does. It requires extensive energy to separate it from water in the reactors in which it's made, and it corrodes engines. Isobutanol and *n*-butanol, by contrast, carry more energy than ethanol does, separate naturally from water, and are noncorrosive.

Last year, researchers led by chemical engineer James Liao of the University of California, Los Angeles (UCLA), transferred a suite of genes into *Escherichia coli*, enabling the bacterium to convert sugars into isobutanol. The remodeled *E. coli* happily churned out isobutanol. But to do the job, it needed to continuously munch sugar, which would add an additional cost to any future commercial version of the technology.

At the meeting, Liao reported that he and his colleagues have now transferred similar isobutanol-making genes into cyanobacteria, blue-green algae capable of photosynthesis. For now, Liao's cyanobacteria produce only

tiny traces of isobutanol. But the UCLA team has a proven record of boosting the output of desired molecules from other microbes, and it is already exploring ways to increase the isobutanol output in cyanobacteria. "It's definitely worth pursuing," says chemical engineer Shang-Tian Yang of Ohio State University in Columbus. "If someone can make it happen, it will be a big breakthrough."

Yang's team reported progress of its own. Microbes don't naturally make isobutanol. But one, a strain of *Clostridium*, makes *n*-butanol, a close chemical relative that is widely used in industry and is being considered as a biofuel. *Clostridium* has proven difficult to engineer and is a slow grower. But at the meeting, a member of Yang's team reported that by growing the bugs in a specially designed bioreactor, they had evolved a strain able to produce 30 grams per liter of *n*-butanol, roughly twice the output of the organisms currently used to make the compound commercially.

"That's impressive," says Huimin Zhao, a bioengineer at the University of Illinois, Urbana-Champaign. Yang says he hopes that by comparing the new *Clostridium* strain with the previous one, his team will learn what makes the new strain so much better at producing *n*-butanol and then use that information to further engineer the microbe. But Zhao notes that few metabolic-engineering tools have been developed to work with *Clostridium*. So Zhao's group and others are now working to reengineer yeast to make *n*-butanol instead of ethanol, which it readily produces. If researchers succeed in turning yeast or cyanobacteria into second-generation fuel producers, ethanol's days atop the biofuel heap may be waning.

—R.F.S.

team has ideas for boosting the efficiency sharply. "If we can do that, it would be a big deal," Swiegers says.

In photosynthesis, plants use chlorophyll and other molecules to capture sunlight. The energy excites electrons, which a cube-shaped calcium-manganese-oxide catalyst uses to split water into molecular oxygen, which it releases, and hydrogen ions (protons) that are used to generate chemical energy for the plant.

For several years, researchers led by Charles Dismukes, a chemist at Rutgers University in Piscataway, New Jersey, have toiled to make a synthetic version of the natural manganese catalyst. That has been a challenge because the version in plants uses a complex protein to stabilize the manganese atoms in their cubical shape. Dismukes's team reported making similarly structured mimics several years back and incorporated them into a proton-conducting membrane 2 years ago.

In their current work, the researchers integrated their catalyst-impregnated membrane into a dye-sensitized solar cell (DSSC). In such cells, sunlight captured by an organic dye excites an electron that is injected into neighboring titanium dioxide nanoparticles and ultimately

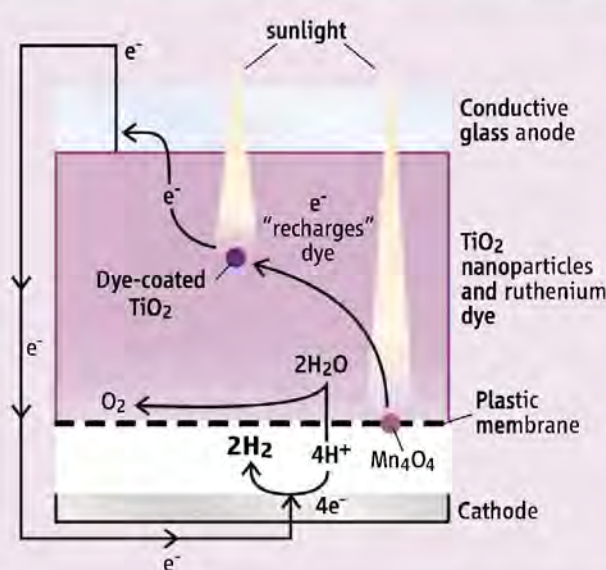
generates an electric current. Earlier this year, researchers led by chemist Thomas Mallouk of Pennsylvania State University, University Park, took this setup one step further by adding an iridium oxide catalyst, which used the excited electrons in DSSC to split water. But iridium is rare and expensive, and the catalyst needs an added electric current to carry out its water-splitting task.

Swiegers, who teamed up with Dismukes and researchers led by Leone Spiccia of Monash University, Clayton, in Australia,

reported at the meeting that the manganese catalyst in DSSC splits water without any added juice. Manganese is also abundant and nontoxic. The setup has two electrodes in water, separated by a plastic membrane that allows protons to pass in one direction. At the anode, sunlight is absorbed by a ruthenium dye, which injects excited electrons into neighboring particles of titanium dioxide; the electrons then flow into an external circuit. The manganese catalyst also absorbs sunlight, in this case grabbing electrons from water molecules and passing them to the dye molecules to restore their light-harvesting ability. When stripped of electrons, the water molecules dissociate into molecular oxygen and protons. The protons pass through the plastic membrane to the cathode, where they combine with electrons from the external circuit, generating molecular hydrogen.

Swiegers says the setup must turn out about 15 times more hydrogen to compete with conventional fuels. But he adds that Dismukes's team thinks a key to achieving that boost is to pack more catalyst clusters around the anode while bringing them into contact with the dye molecules. If the researchers pull off that trick, plants may be in for a bit of competition in the sunlight-to-fuels business.

—ROBERT F. SERVICE



Sunlight to fuel. In a dye-sensitized solar cell, a manganese catalyst splits water into oxygen (O_2) and protons (H^+), which form hydrogen gas (H_2).

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LETTERS

edited by Jennifer Sills

Teaching and Learning Strategies That Work

FOR MORE THAN FOUR DECADES, WE HAVE TAUGHT CHEMISTRY. AS WE STRUGGLED TO BECOME better teachers, we developed (and borrowed) a number of effective strategies. Learning and teaching are a double flame—each feeds the other. We begin with some suggestions for instructors.

Foster the mentor-apprentice bond you have with your students. Once established, this relationship helps students learn in two ways: First, the student admires the mentor and wants to attain the mentor's level of understanding. Second, the mentor can help the learner navigate boring or tough stages on the way to mastery.

Teach students how to learn. Students may not realize that learning progresses through stages, with memorization being only an early one (1). Bloom and colleagues identified levels of learning, now labeled as remembering, understanding, applying, analyzing, evaluating, and creating (2, 3). Making students aware of different kinds of learning can transform them from rote memorizers into independent, self-directed learners (4). We have also found that when students learn about metacognition (thinking about your own thinking) (5) they change their attitudes about learning and begin to implement effective study strategies.

Grade on a combination of dominant absolute performance (examinations and quizzes) and minor "curved" components (such as labs). A clearly defined contract is best, in which performance at a certain level ensures a grade, with adjustments only to increase the grade. With this system, students are empowered—the outcome of their course grade is dependent on their work alone rather than their work relative to the work of others. For this to be effective, you will need to construct exams for which the level of mastery of the material is accurately reflected by the grade (6).

Do as many demonstrations as possible. Demonstrations are somewhere between magic and science (7), and they cross the bridge from entertainment to learning. Ideally, they also incorporate content and thus enhance learning.

We disagree about "cheat sheets"—allowing each student to bring to a test one page on which he or she can write anything. On one hand, the sheet serves as a learning tool; in composing it, the student organizes what he or she has learned. On the other hand, students may spend time looking for information to copy onto the sheet instead of working to understand concepts.

More generally, let these ideas shape your teaching: (i) Empathy. Students will respond when they know that you genuinely care about them. (ii) Active learning. Student participation will facilitate learning. (iii) Judicious interplay of groups and individuals. Learning is a solitary activity, yet it can be enhanced by group work. (iv) Empowerment. Encourage students to feel that they are responsible for their own learning successes.

We have found that students can improve their learning through the following strategies:

Take notes by hand, even if the class notes are provided. As soon as possible, condense and extend the notes, paraphrasing them into your own words. Taking notes is active engagement, which is imperative for learning (8). The process of paraphrasing notes helps transfer information from short-term to long-term memory (9, 10).

If you miss a class, get notes from a fellow student instead of downloading them from a Web page. Discussing class notes facilitates learning, both for the student who asks questions about the notes, and for the student who engages in teaching by answering the questions.

To maximize learning from homework problems, first study the text and lecture information relevant to the problems. Next, work through the problems without looking at an example or the solutions in a solutions manual. Finally, compare your approach—not just your answer—to the text's. (Instructors should always provide ways to work through each problem, not just the answers.) Focusing on methods, rather than final answers, helps you develop agile, flexible thinking.

To make the most of group learning, the alone-together-alone sequence is crucial. First try to do the homework problems or prepare for the exam alone. Then, access the collective wisdom of a group, watchful for the pitfalls of group dynamics. Finally, return to solving the problem set or facing the exam on your own. Social constructivist learning theorists have shown that meaningful learning results from study groups with two crucial features: discussion and problem-solving activities (11). Tips for forming effective groups are available (12).

Individually and in groups, make up practice tests when preparing for examinations. This exercise involves the selection and organization of all the material and fosters discussion of what material is important enough to be on the test. This is the only way to get into the teacher's mind.

Finally, we provide a suggestion for both teachers and students:

Recognize that students have different learning styles. Learning style can refer to a person's preferred modality [visual, auditory, verbal, or kinesthetic (13)], Myers-Briggs personality type (14), or other learner characteristics. People do disagree about these (15), but we think they are useful. Students should work to understand their individual learning preferences in order to become more efficient learners. Teachers need to recognize that there are different ways to learn and try to accommodate a variety of learning styles in their classes. Instructors should resist the temptation to teach only as they were taught or in a manner that suits their own learning style.

Our suggestions are not prescriptive; we just want to share with you some of the strategies we



Sandra Y. McGuire



Roald Hoffmann

have improvised and developed over the years to facilitate learning for, rather than to deliver instruction to, the students we have taught. We hope that you will find them useful tools in your teaching and in your students' learning (16).

ROALD HOFFMANN^{1*} AND SAUNDRA Y. MCGUIRE²

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Science-Savvy Physicians

IN THEIR EDITORIAL ("SCIENCE FOR FUTURE physicians," 5 June, p. 1241), S. Long and R. Alpern mention the release of a major report on medical education arguing for increased emphasis on basic scientific competence. However, they fail to note the report's relevance to the broader arena of biomedical science and innovation. Beyond the obvious need for clinicians to understand the scientific underpinnings of disease mechanisms when treating patients, a sound scientific education is critical to training physicians who translate research findings to a clinical setting.

A key factor fueling these new recommendations is the desire for more students to enroll in physician-scientist (M.D./Ph.D.) programs in the United States. A commonly held belief is that these physician-scientists will use both their clinical and scientific training to facilitate the translation of discoveries to the clinic. More often, physician-scientists either become pure clinicians or focus all of their energies on basic sciences, thus playing the same role as Ph.D.-trained biomedical scientists (1).

Whether due to time constraints or intent, physician-scientist programs have generally failed to fulfill this dual role and have focused excessively on basic research. This creates unnecessary redundancy and also discourages and even deceives medical students interested in more applied research. While I support the findings of this report, maximal value from the recommendations can only be derived by training and allowing physicians with a sound scientific foundation to play a more downstream and enabling role in facilitating the delivery of innovation to the clinic.

Recently, several medical schools in the United States and internationally, including the University of Toronto, have recognized this

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trend. In response, they are revamping traditional clinical investigator programs and creating interdisciplinary team-based training programs in their place. The new programs bring together residents or M.D./Ph.D. students, biomedical engineers and scientists, and entrepreneurs to systematically generate innovations for surgery, imaging, and regenerative medicine, and then take them to the clinic.

Physicians with a strong basic scientific foundation can play a facilitating role in reconciling the science behind the prototypes, assays, or discoveries with the disease mechanisms underlying the clinical needs. This role is particularly important for global health applications, where tailored user-driven development and delivery is often more challenging than the actual discovery itself. Physicians need not actually be the ones discovering—leave that to the scientists. The recent development of several Ph.D. programs that include “rotations” in the clinic further relieves the need for inefficient M.D./Ph.D. training programs to train basic scientists with clinical understanding.

By optimizing the contribution of physicians through collaboration with scientists and engineers, such interdisciplinary pro-

grams can help to defuse the myth of medical innovation and practice as being an “art”—a myth that contributes to the perception among physicians that basic scientific competency is unimportant. What we need are not more physician-scientists, but physician-innovators and physician-facilitators. **JUSTIN CHAKMA**

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A Step Toward Unification

CONTRARY TO THE VIEW OF BIODIVERSITY INFORMATICS (BI) depicted in the News of the Week story “Biodiversity databases spread, prompting unification call” (C. Thomas, 26 June, p. 1632), substantial progress has been made in the past few years, particularly since the advent of the Global Biodiversity Information Facility (GBIF) in 2001. GBIF is not a project, as depicted in the News story, but a multi-country agreement to establish an international organization, modeled on other international treaties. An initial 17 countries (mandated through their

Science Ministries) negotiated the establishment of GBIF as a commonly owned and commonly funded mechanism to meet their common BI challenges. The Memorandum of Understanding that each country signs commits them to contributing financially and to supporting free and universal sharing of their data. As such, GBIF has a sustainable funding model to support an international secretariat and implementation of an agreed work program as well as the mandate to build the infrastructure needed to make biodiversity data freely accessible to all through the Internet.

Through various community-owned initiatives and partnerships, GBIF has overcome many of the BI challenges highlighted in the News of the Week story as still problematic, including the development of global standards, data-sharing protocols, best practices in areas such as digitization and data cleansing, and increasingly, in developing metadata standards and registries, ontologies, and spatial analysis applications. GBIF is admittedly still a work in progress, yet it provides the international foundation through which solutions to these complex problems can be developed. In terms of collaboration and interoperability, GBIF has a

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growing membership of 51 countries and 42 international organizations (most of whom were represented at the e-Biosphere conference) and already provides access to over 180 million primary biodiversity records in more than 7600 data sets from more than 265 institutions around the world. This is undoubtedly a unification success story, albeit with much still to be done.

NICHOLAS KING* AND DAVID PENMAN

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Letters to the Editor

Letters (~300 words) discuss material published in *Science* in the previous 3 months or issues of general interest. They can be submitted through the Web (www.submit2science.org) or by regular mail (1200 New York Ave., NW, Washington, DC 20005, USA). Letters are not acknowledged upon receipt, nor are authors generally consulted before publication. Whether published in full or in part, letters are subject to editing for clarity and space.

TECHNICAL COMMENT ABSTRACTS

COMMENT ON "Energy Uptake and Allocation During Ontogeny"

Anastassia M. Makarieva, Victor G. Gorshkov, Bai-Lian Li

We demonstrate that the model of energy allocation during ontogeny of Hou *et al.* (Reports, 31 October 2008, p. 736) fails to account for the observed elevation of metabolic rate in growing organisms compared with similarly sized adults of different species. The basic model assumptions of the three-quarter power scaling for resting metabolism and constancy of the mass-specific maintenance metabolism need to be reassessed.

Full text at www.sciencemag.org/cgi/content/full/325/5945/1206-a

COMMENT ON "Energy Uptake and Allocation During Ontogeny"

Tânia Sousa, Gonçalo M. Marques, Tiago Domingos

Hou *et al.* (Reports, 31 October 2008, p. 736) presented a model for energy uptake and allocation over an organism's growth and development. However, their model does not account for allocation to reproduction (essential to adults) and growth without assimilation (essential to embryos) and is therefore only applicable to organisms growing with abundant food in the juvenile stage.

Full text at www.sciencemag.org/cgi/content/full/325/5945/1206-b

RESPONSE TO COMMENTS ON "Energy Uptake and Allocation During Ontogeny"

Wenyun Zuo, Melanie E. Moses, Chen Hou, William H. Woodruff, Geoffrey B. West, James H. Brown

Our extended ontogenetic growth model is a theoretical model based on conservation of energy and general biological mechanisms underlying ontogenetic growth. We do not believe that the comments of Makarieva *et al.* and Sousa *et al.* expose substantive problems with our model. Nevertheless, they raise interesting, still unresolved questions and point to philosophical differences about the role of theory and of simple, general models as opposed to complicated, specific models.

Full text at www.sciencemag.org/cgi/content/full/325/5945/1206-c

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MEDICINE

Evolutionary Biology for Doctors

Peter T. Ellison

In his 1870 address to medical students at University College, London, Thomas Huxley—already known as “Darwin’s bulldog” and fast becoming the most important voice in Britain on all policy questions regarding science and education—did not mention evolution even once. In fact, far from suggesting the inclusion of evolution in medical curricula, he advocated removing unnecessary topics, including his own beloved field of comparative anatomy. There was simply too much information medical students needed to absorb from the crucial areas of physiology, pathology, and pharmacology for broad topics such as evolutionary biology to be covered.

In 2009, the 150th anniversary of the publication of *On the Origin of Species*, evolutionary biology is still trying to earn a place in medical education. The core competencies recommended by a recent joint committee of the Association of American Medical Colleges and the Howard Hughes Medical Institute on the scientific knowledge required by future physicians include an understanding of evolution by natural selection (1). At an April meeting, “Evolution in Health and Medicine,” sponsored by the National Academy of Sciences and the Institute of Medicine, a panel of deans and faculty from leading medical schools around the world endorsed the incorporation of evolutionary principles in medical curricula (2). And yet one can probably count on the digits of a three-toed sloth the number of medical schools currently offering such instruction.

Part of the problem is still the crowded medical curriculum that Huxley recognized. But in the era of genomics and proteomics, this no longer seems an adequate excuse. More problematic may be the lack of appropriately skilled faculty members to teach evolutionary principles and the lack of appropriate materials from which to teach. In the 14 years since Randolph Nesse and George Williams published *Why We Get Sick* (3), a number of books devoted to Darwin-

ian medicine have appeared. But they have either been edited compendia of recent research that assume an understanding of evolution or popular reads for a lay public. *Principles of Evolutionary Medicine*, by Peter Gluckman, Alan Beedle, and Mark Hanson (authorities on the developmental origins of health and disease), is the first specifically designed as a textbook appropriate for medical students and medical schools, and it succeeds brilliantly.

The authors attain their goal in large part because they neither assume too much prior knowledge nor underestimate the capacity of their readers to absorb copious and sophisticated material at a rapid rate. They also succeed because the book is clearly written and wonderfully organized. The first of the book’s three sections builds up an understanding of evolutionary theory from ground zero to a sophisticated, contemporary level. The authors introduce readers to life history theory, game theoretic approaches to evolutionary dynamics, and intragenomic conflict, to name a few of the current topics covered. They review human evolutionary history in sufficient detail for readers to appreciate specific adaptations in the hominin lineage. Side boxes present compelling examples drawn from current research literature that convey the excitement and relevance of research in human evolutionary biology.

In the second section, four chapters showcase the power of evolutionary biology to organize and explain complex areas of human biology relevant to modern medicine. The first, discussing human reproduction, interweaves principles of developmental biology, life history biology, and aging using

an evolutionary understanding of reproductive physiology and the forces that have shaped it. Contemporary medical issues including advancing puberty, fertility and infertility, menopause, and reproductive cancers are presented in new light. The second considers nutrition and metabolism, topics particularly pertinent in a time of increasing obesity and prevalence of metabolic syndromes that also demonstrate the potential of an evolutionary perspective in forging a synthetic understanding of genetic, developmental, environmental, and behavioral risk factors. The chapter on defense covers such topics as the evolution of virulence, antibiotic resistance, immunization strategies, and autoimmune disease. The fourth chapter discusses social organization and human behavior, including mental illness as well as the influence of lifestyle factors on human health and disease.

The final section presents a set of effective organizing principles for understanding the causes of human diseases from an evolutionary perspective. The authors provide a satisfying list of categories of explanation that medical students and professionals can internalize and readily apply to other areas of their education and experience.

Those who have been immersed in evolutionary medicine and related topics may find things to criticize in this text.

Their gripes, like mine, are likely to be particular and rather petty: favorite topics slighted, controversial interpretations presented less critically than one would like, etc. But my own complaints evaporate when I realize that *Principles of Evolutionary Medicine* brings students to a point where they can meaningfully engage in debates on the issues at a fairly sophisticated level.

The mass of material that successful medical students must master has probably multiplied many times since Huxley’s day. Evolutionary biology, however, is no longer an expendable topic in medical education. The task for medical schools is to figure out how to teach it—a task that has just been made much easier.

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10.1126/science.1179152

Principles of Evolutionary Medicine

by Peter Gluckman, Alan Beedle, and Mark Hanson

Oxford University Press, Oxford, 2009. 312 pp. \$130, £65. ISBN 9780199236381. Paper, \$65, £32.50. ISBN 9780199236398. www.evomedicine.org

ATMOSPHERE

More Than Just Talking

Roger Turner

“Everybody talks about the weather, but nobody does anything about it.” Desperate writers have long leaned on Charles Dudley Warner’s supposed quip to start articles, often attributing it to Mark Twain. But Bernard Mergen’s boisterous *Weather Matters* illustrates how talking is itself a kind of doing. Cultural discourse is our oldest and most effective technique for managing the weather; ideas and habits determine the weather’s impact on our lives as much as air masses and jet streams do. Romping through a century of art, literature, humor, journalism, and science, Mergen shows how Americans have made the weather by talking about it.

This serious but implicit message emerges from a delightful collage of anecdotes and quotations. On one page Mergen recovers a forgotten debate about the discomfort index, a combined measure of temperature and humidity introduced in the late 1950s. A Jesuit magazine urged the Weather Bureau to desist, worrying that people less sensitive to heat would feel guilty that they did not experience the misery they were entitled to, leading to “a nation of thermohumidipaths.” More cheerfully, Mergen shares the work of Victor Hernández Cruz, whose poem “Problems with Hurricanes” explains why it’s not the wind or the water you should worry about, but the fruit:

How would your family
feel if they had to tell
The generations that you
got killed by a flying
Banana.

With long practice as a literary scholar, Mergen deftly unpacks the subtler meanings of passages like these without bruising their humor. His smooth prose organizes an astonishingly diverse collection of sources into a flowing history of American weather discourse. In just two pages at the start

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of his discussion on visualizing clouds, he fluidly connects the Romantic era cloud systematizer Luke Howard, the invention of the nephoscope, NASA’s CloudSat, and the late-19th-century art critic and nature writer John Van Dyke. Elsewhere he discusses Boy Scout manuals, the motion picture *Twister*, a report by President Franklin Roosevelt’s Science Advisory Board, the 1917 children’s book *The Boy with the U.S. Weather Men*, and poet laureate Ted Kooser. Despite the volume of this discourse, Mergen does not believe Americans are obsessed with weather. Some people certainly are, but many more obsess over “genealogy, NASCAR, or sex.” Rather, we are possessed by weather; it surrounds us and permeates our art, language, and science.

Although the author acknowledges that the “popularity of weather as a hobby, a business, and a political issue has grown exponentially in the past few years,” the book’s structure and organization emphasize the continuities of American weather culture rather than its changes. Three themes run through Mergen’s study: perceiving, marketing, and managing the weather. Each of these themes emerges in various cultural forms. Perception includes writing storms into poems as well as simulating them on

computers, mapping them on television, and filming them for Hollywood. It’s easy to see the atmosphere marketed on The Weather Channel and in advertisements for Florida’s sunshine, but Mergen also perceptively explicates how recurrent efforts to advertise

meteorological expertise sell the National Weather Service’s authority to a persistently skeptical public.

In this era of anthropogenic climate change, distinguished scientific voices are again clamoring to be allowed to do something about the weather. The cultural history of efforts to physically

manage the weather, Mergen shows, ought to give a pause to would-be geoengineers. Like historian of science James Fleming (1, 2), Mergen sees a record of social as well as technical challenges in the history of “designer weather.” Cloud-seeding experiments from the 1950s to the 1980s revealed not only how poorly scientists understood cloud dynamics but also the many interests holding stakes in different weather. Mergen quotes E. B. White:

If, as the rainmakers would have it, man does invade the sky and nudge clouds, his flight will, I predict, be but the beginning of such practices, and we shall find the makers of lightning also aloft, to satisfy the desires of manufacturers of lightning rods, who may decide that lightning is in short supply and devise a way of setting more of it loose.

The need to manage conflicting interests will only be magnified if intentional climate modification becomes possible on a planetary scale. In 1938, Weather Bureau meteorologist T. A. Blair imagined weather control 100 years hence. The atmosphere’s interconnections meant that “while the one area is getting the kind of weather it wants, another region is subject to unfavorable weather.” Writing on the eve of World War II, Blair foresaw, “International complications begin and the human masses of the world are plunged into a war for the control of the air masses.”

Mergen’s own outlook is never so bleak. Instead, with a light touch and plenty of humor, he suggests that our talk about the weather reveals as much about ourselves as it does about the clouds around us.

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10.1126/science.1174857

CREDIT: TIME



Management guru. Langmuir used his Nobel laureate status to promote large-scale weather control.

AGRICULTURE

Agricultural Research, Productivity, and Food Prices in the Long Run

Julian M. Alston,^{1*} Jason M. Beddow,² Philip G. Pardey^{2†}

A reinvestment in agricultural R&D is critical to ensuring sufficient food for the world in the coming decades.

In a recent update of earlier estimates (1), the Food and Agriculture Organization (FAO) of the United Nations reported that more than one billion people now suffer malnutrition (2). Despite declines in food prices from their 2008 highs, local prices in many developing countries are still high by recent historical standards. Long-run trends in global food commodity prices are driven by differential rates of growth in the supply and demand for food crops, feed, and livestock products.

Growth in demand for agricultural commodities largely stems from growth in demand for food, which is driven by growth in population and per capita incomes (especially the economic growth of the fast-growing economies of Asia), coupled with new demands for biofuels. Growth in supply of agricultural commodities is primarily driven by growth in productivity, especially as growth in the availability of land and water resources for agriculture has become more constrained. Thus, agricultural productivity growth will be a pivotal determinant of long-term growth in the supply, availability, and price of food over the coming decades.

Here, we document a slowdown in growth of agricultural productivity and grain yields. If this slowdown in productivity persists, it could have profound implications for food price trends in the future.

Global Crop Yields and Productivity

Global yields for maize, rice, wheat, and soybeans (in metric tons per harvested hectare) grew rapidly from 1961 to 2007: Maize and wheat yields each grew by a factor of 2.6, while rice and soybean yields increased by a factor of 2.2 and 2.0, respectively (3). However, for all four crops, in both developed and developing countries, rates of yield growth were slower during 1990 to 2007 than during

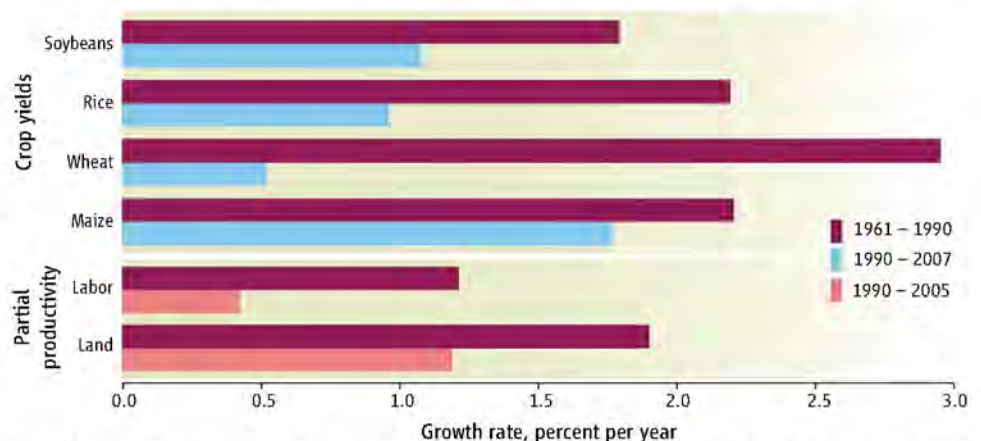


1961 to 1990 (see graph, below, and table S1). A slowdown in crop yield growth was seen in more than half of the countries that grew these four crops. More critically, compared with all producing countries, a higher proportion of the top 10 producing countries experienced a slowdown for all four crops (4).

Global land productivity, reflecting worldwide output of 185 crop and livestock commodities per harvested and pastured area, was 2.4 times in 2005 what it was in 1961 (equivalent to growth of 1.96% per year). Labor productivity, the output per agriculture worker, grew by a factor of 1.7 during that span (1.20% per year growth) (table S2). These productivity develop-

ments reflect relatively slow growth in the use of agricultural land and labor (0.31% and 1.07% per year, respectively), compared with growth in global agricultural output (2.27% per year) (table S2).

In parallel with global crop yields, global land productivity grew at a slower pace from 1990 to 2005 (1.82% per year) than from 1961 to 1990 (2.03% per year) (table S2). Labor productivity increased at a faster rate from 1990 to 2005 than from 1961 to 1990 (1.36% versus 1.12% per year, respectively) (table S2). These world totals are influenced by the significant, and in many respects exceptional, case of China, where land and labor productivity growth has accelerated

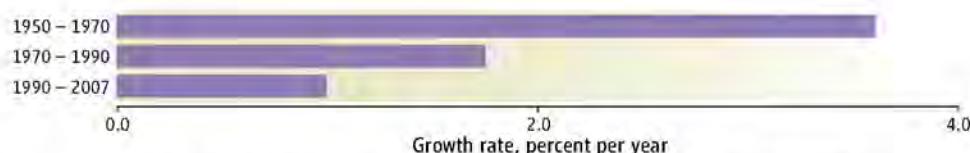


Global yield and agricultural productivity growth rates, percent per year for 1961 to 2007. Yield is measured as metric tons per hectare. Labor and land productivity are total agricultural output per agricultural worker and agricultural area, respectively, excluding China. Total agricultural output was derived using 1999 to 2001 price weights. Authors' calculations are based on data from (4). See notes accompanying table S1 and table S2.

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Percent annual growth of U.S. public agricultural R&D spending for 1950 to 2007. The underlying public agricultural R&D spending data are adjusted to reflect 2000 prices. Public agricultural R&D includes intramural USDA research and research conducted at the state agricultural experiment stations. Extracted and compiled by the authors using (15–17).

recently (5). If China is left out, global land and labor productivity growth has been substantially slower since 1990 than during the previous three decades (see graph, p. 1209, and table S2). Among the top 20 producing countries (according to their 2005 value of agricultural output), land and labor productivity grew substantially more slowly from 1990 to 2005 than from 1961 to 1990, again, once the case of China is set aside.

Agricultural Research and Development

Many factors may have contributed to the slowdown in agricultural productivity growth. Changes in weather or climate, land degradation, shifts of the location of production to less favorable environments, farmer responses to resource scarcity or higher prices of inputs, changes in public institutions [e.g., in China and the former USSR (5–7)], and evolving pests and diseases may all have contributed.

Agricultural R&D also is an important element of the story, a critical policy instrument that governments can apply to influence the path of agricultural productivity. Organized public and private investment in agricultural R&D was a primary driver of the comparatively rapid growth in agricultural productivity experienced in the latter half of the 20th century (8, 9). The interactions are complex, with long and uncertain time lags between initial investment in research and realization of the returns. But although it takes a long time, perhaps decades, for R&D to affect productivity, it then typically affects productivity for decades more. These effects may be subtle. Much investment in agricultural R&D is of a “maintenance” type, designed not to increase yields so much as to prevent yields from declining in the face of coevolving pests and diseases or other environmental changes (10).

Despite the long lags, hundreds of cost-benefit studies have reported that investments in agricultural R&D have yielded high returns (8, 9, 11). Such studies have indicated that the world has persistently underinvested in agricultural R&D (8, 11, 12) and have been cited by economists to justify an increased rate of growth in agricultural R&D spending (8), which may help restore pro-

ductivity growth and ameliorate hunger and poverty (12). Instead, we have seen a slowdown in the growth rate of public agricultural R&D investments (see graph, above) and a change in the balance between private and public investments to increase the private share. Moreover, funds have been redirected away from farm productivity toward other concerns, such as the environmental effects of agriculture; food safety and other aspects of food quality; and the medical, energy, and industrial uses of agricultural commodities (fig. S1). For example, in 1975, an estimated 66% of all research conducted by the state agricultural experiment stations in the United States was directed to maintaining and enhancing farm productivity; by 2007, this share had slipped to 57%.

Data for other developed countries show patterns somewhat consistent with those in the United States (13). In the latter half of the 1990s, public agricultural R&D was massively reduced in Japan and also, to a lesser degree, in several European countries.

In the past, most countries (especially the poorest ones) have relied heavily on spillovers of knowledge and technology resulting from agricultural R&D undertaken by a small number of developed countries. Thus, a continuation of recent trends in funding, policy, and markets is likely to have significant effects on long-term farm productivity for food staples in developed and developing countries alike (12). A revitalization of agricultural R&D investments in developed countries can be justified on narrow cost-benefit criteria (12). In addition, it will contribute to the global public good by restoring and sustaining productivity growth over the long run, which in turn will mitigate hunger and poverty and, at the same time, reduce pressure on the natural resource base.

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herein was developed by the authors drawing from (4, 14) for yield and productivity estimates and (15–17) for trends in U.S. agricultural R&D. For any given year and geographic unit in question, data reflect only one observation. Total growth of yields, productivity, and R&D expenditures over multiple-year periods (the difference between end-year and initial-year observations) is expressed as annual rates to allow comparisons between periods of different lengths. The nature of these data (i.e., single-valued, not averages calculated from samples) limits reporting of additional statistics. See supporting online materials for additional data and details.

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Supporting Online Material

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ASTRONOMY

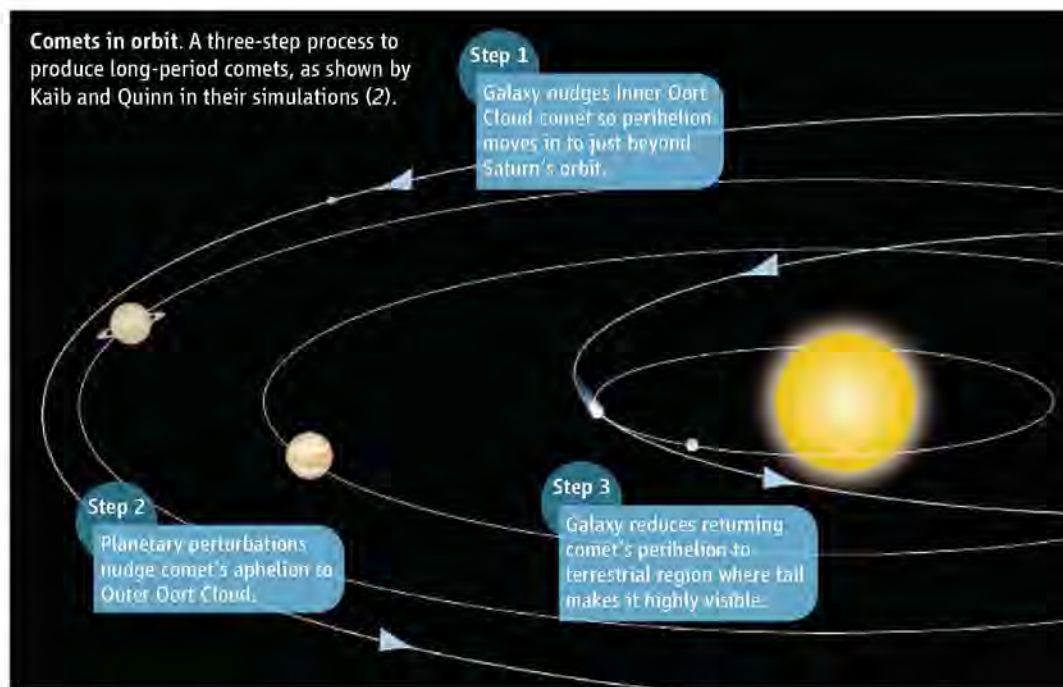
Re-viewing an Old Comet Reservoir

Martin Duncan

A few times a year, new long-period comets (LPCs) on elongated orbits come to within ~ 1 astronomical unit (AU) of the Sun (1 AU is the distance between the Earth and the Sun), where they draw our attention by releasing majestic tails of dust and ions from their frozen surfaces. The original orbits typically trace back out to distances of at least 20,000 AU. Since Jan Oort's classic 1950 paper (1), it was believed that the reservoir for the LPCs is a roughly spherical "Oort Cloud" of approximately 1 trillion comets, which extends from 20,000 to 100,000 AU (about halfway to the nearest stars). The bodies in the Oort Cloud are thought to be the surviving population of unincorporated remnants of planetary building blocks that were gravitationally scattered outward by the growing planets. On page 1234 of this issue, Kaib and Quinn (2) present results of a simulation that suggest that a substantial fraction of LPCs (perhaps the majority) are stored in an "inner" Oort Cloud reservoir considerably closer to the Sun than expected, at distances of around 3000 to 10,000 AU.

The inner Oort Cloud was not previously thought to produce many LPCs, for the following reason. If the Sun were the only massive body in the universe, every gravitationally bound comet would orbit once per period on a fixed elliptical path moving from the same perihelion distance (closest to the Sun) to the same aphelion distance (farthest from the Sun). However, the gravity of the plane of our Galaxy (which we see as a nighttime band called the Milky Way) exerts a slightly different nudge on the comet than on the Sun, especially when the former is near aphelion. Although this does not appreciably change the aphelion distance, it does change the comet's perihelion distance for its next passage near the Sun. In this way a tiny fraction of bodies from the Oort Cloud at any given time have their perihelion distances drawn into the realm of the planets (3). However, the massive planets Jupiter and Saturn exert strong kicks on comets with perihelion distances near their orbits (~ 5 to 10 AU). To be seen as an LPC with perihelion near Earth, the comet's perihelion distance must jump over the region from ~ 15 AU down to ~ 1 AU in one

Comets in orbit. A three-step process to produce long-period comets, as shown by Kaib and Quinn in their simulations (2).



orbital period. Galactic perturbations are only capable of this rapid perihelion reduction for comets with aphelion distances greater than about 40,000 AU, i.e., only for objects in the outer Oort Cloud.

However, the body need not have been in the outer cloud for the entire age of the solar system—another route is possible (4). The object might have spent most of its life in the inner cloud and very recently been nudged into its current (large aphelion) orbit by weak planetary perturbations when its perihelion was drawn in to just beyond the orbit of Saturn (see the figure). The simulations of Kaib and Quinn show that a large fraction of the LPCs are generated via this mechanism.

The results of these simulations not only change our view of where the progenitors of LPCs are stored but may also provide constraints on models of planet formation and on the properties of the stellar nursery in which the Sun spent its formative years (5). The existence of a relatively massive inner cloud containing comets with relatively small aphelion distances could indicate that the Sun formed in a relatively dense star cluster. Indeed, the otherwise baffling orbit of the recently discovered (6) large body named Sedna (perihelion near 80 AU and aphelion near 1000 AU) is plausibly explained by just such a scenario (7).

The new results are comforting in another way. Although it is difficult to accurately

Simulations show that the inner Oort Cloud is the source of many more long-period comets than expected.

determine the mass of the outer Oort Cloud, some of the higher estimates conflict with current planet-formation models in which the cloud formed while the Sun was in its current low-density galactic environment (8). However, models of comet cloud formation during the star cluster phase show that capture of scattered objects into the cloud is much more efficient (9, 10). Kaib and Quinn's predictions of the inner cloud masses are broadly consistent with current planet-formation models.

On the theoretical side, these tantalizing results will encourage more complete beginning-to-end simulations of Oort Cloud formation and dynamical evolution. Such models can be observationally tested in the near future, as they will make specific predictions for the flux and orbital element distributions of comets with perihelia in the region of the giant planets. Deep wide-field observational surveys such as Pan Stars (11) and the Large Synoptic Survey Telescope (12) should soon be providing extensive data on such objects. In the longer term, current ground-based (13) and proposed space-based (14) techniques will search for the brief modulation of the light of background stars as Oort Cloud comets pass between the stars and the observer, thereby diffracting the light (15). This could provide estimates of the cometary distributions all the way to the outer Oort Cloud.

Given these exciting prospects, the future is bright for studying the faint outer limits of our solar system.

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EVOLUTION

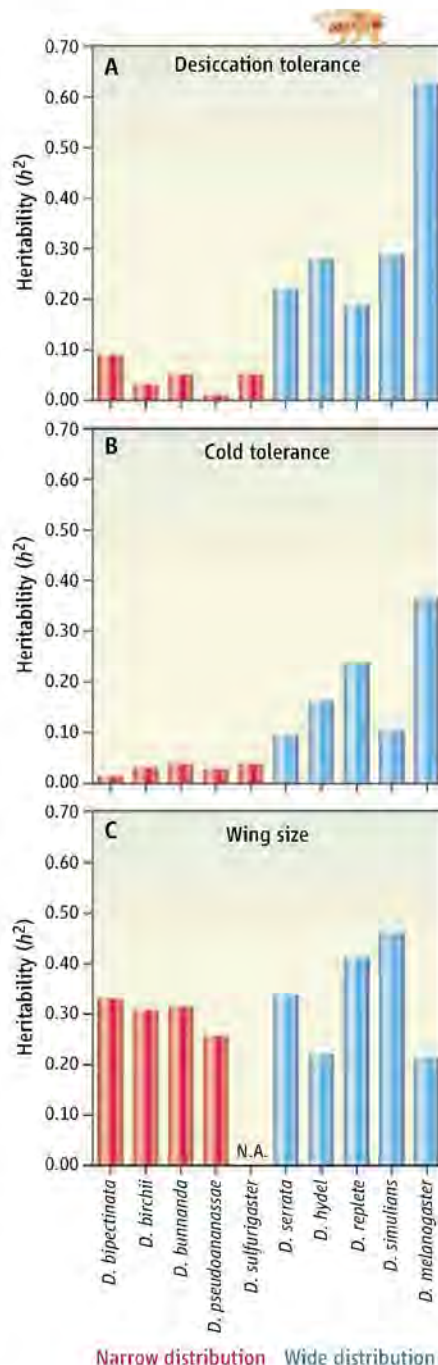
Genetic Constraints on Adaptation?

Juha Merilä

According to widely held perception among evolutionary biologists and geneticists, most traits in wild animal and plant populations are heritable, and are thereby able to respond to natural selection. Hence, adaptation and evolution should not be limited by a lack of genetic variability, except on rare occasions (1). On page 1244 of this issue, Kellermann *et al.* (2) challenge this view, reporting low levels of genetic variation in cold and desiccation tolerance in tropical *Drosophila* species with narrow geographic distributions. In contrast, widely distributed *Drosophila* species show much higher levels of genetic variability in the same tolerance traits.

These findings suggest—but do not prove—that an absence of genetic variability in key tolerance traits may limit species distributions. This is a novel extension of the climatic variability hypothesis (3), which has been proposed to explain the tendency of species distributions to become narrower toward the tropics (3). Given the generally narrow physiological tolerance range of tropical ectothermic animals (4, 5), the results provide yet another reason to worry about the fate of tropical ectotherms in the face of changing climatic conditions.

Impacts of climate warming for ectothermic animals are expected to be more severe in tropical than in temperate regions (4–6), even though the rate of climate warming in the tropics is lower than at higher latitudes. This is because a shift of a given magnitude in ambient temperature is expected to push more tropical than temperate ectotherms outside of their tolerance limits (4, 5). Add to this the prospect—as suggested by the *Drosophila* data of Kellermann *et al.* (see the



A lack of genetic variation in ecologically important traits may limit the ability of tropical species to respond to climate change.

figure)—that the levels of genetic variation in tolerance traits are also lower in the tropics than in temperate regions, and the future for tropical biodiversity looks bleak. Low levels of heritable variation in tolerance traits greatly limit the possibilities for adapting to climate change (7, 8).

By bringing genetic variability in ecologically important traits relevant to climatic adaptation into focus, the study by Kellermann *et al.* serves also as a timely reminder about the complexities of hereditary patterns in the wild. Much of the current evolutionary biology research into anthropogenic impacts on wild populations assumes that the traits of interest are heritable, and that observed phenotypic changes reflect genetically based evolutionary changes (8, 9). These assumptions may be unwarranted given the scarcity of relevant genetic data (8–10) and the fact that phenotypic trends do not always mirror underlying genetic trends (11). By demonstrating large and consistent differences in levels of heritable genetic variation between two sets of populations, Kellermann *et al.* highlight how little we actually know about the genetic basis of the variation in ecologically important traits in the wild.

Although the patterns in genetic variability levels uncovered by Kellermann *et al.* are clear, the reasons behind them are not. Why do the narrowly distributed tropical species exhibit lower levels of genetic variability in tolerance traits than do more widely distributed species? Erosion of

Genetic variability in narrowly and widely distributed species. Narrowly distributed tropical *Drosophila* species (red bars) exhibit much lower levels of genetic variability in both desiccation (A) and cold tolerance (B) than do more widely distributed species (blue bars). Levels of genetic variability in wing size (a proxy for body size) (C) do not differ between narrowly and widely distributed species. N.A., not available.

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genetic variation in traits subject to strong directional or stabilizing natural selection (12) is an obvious, but as yet unsupported, alternative (2). However, if the explanation were to lie in population processes—such as inbreeding and genetic drift—then tropical species should also exhibit lowered genetic variance in other traits (such as body size) and in neutral marker genes. Neither expectation was met in this study (see the figure, panel C) (2).

Whatever the reason(s) for differing levels of genetic variation in key tolerance traits in narrowly and widely distributed *Drosophila* species, the message from the

Kellermann *et al.* study is loud and clear: Genetic constraints on adaptive evolution in response to climate warming may be more widespread than previously thought (1, 13). The challenge for future studies in evolutionary physiology (14) is to reveal how general these findings are, and uncover the mechanisms behind the observed patterns.

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10.1126/science.1179326

BEHAVIOR

Like Infant, Like Dog

Michael Tomasello and Juliane Kaminski

Over the past decade, behavioral scientists have uncovered a surprising set of social-cognitive abilities in the otherwise humble domestic dog. These abilities are not possessed by dogs' closest canine relatives, wolves, nor by other highly intelligent mammals such as great apes. Rather, these skills parallel some of the social-cognitive skills of human children. On page 1269 of this issue, Topál *et al.* extend our understanding of these specialized abilities, showing that in some situations, they may lead man's best friend, just as they do young children, into curious errors. (1)

The original experimental task suggesting unusual social-cognitive skills in dogs was a communication task (2, 3). If a piece of food is hidden in one of several opaque cups, and then a human points to the cup containing the food, a dog can infer the location of the hidden food quite readily. To comprehend the pointing gesture in this context, a dog must infer something about why the human is directing its attention to a boring cup—why the human's communicative gesture is relevant to their search for the food. Dogs' skills in this task are surprising because, as simple as it seems for humans [human infants solve it at around the first birthday (4)], even our nearest primate relatives, the great apes, fail at it miserably (5), as do wolves (5, 6). Dogs have not shown special cognitive skills relative to other mammals in nonsocial cognitive tasks, such as understanding space or physi-



Eager to please. Dogs, like human infants, are specially adapted for following instructions from humans.

cal causality (7), whereas they show special social-cognitive skills even as young puppies, before they've had much experience with humans (5, 8). The so-called domestication hypothesis is thus that dogs' specialized skills arose as adaptations for interacting and communicating in the human social environment in which they have lived for more than 10,000 years (5, 6, 9).

The task that Topál *et al.* used is called the object permanence task. For human infants, the result is rather strange. Suppose you hide an object in location A several times, and an infant finds it there each time. If you then hide the object in location B, right in front of its eyes, the infant continues to search for it at location A. Some theorists believe this error

The domestic dog possesses social-cognitive skills that parallel those of human children.

indicates that infants have a profoundly different conception of the world than adults (10). In a previous study, Topál and colleagues (11) proposed a very different explanation—that infants are attending not just to the object but also to the adult performing the hiding operations. Because the adult is calling the infant's attention and showing the manipulations, the infant sees the original hiding act (in location A) as pedagogy from the adult about where this object normally goes. By the time the adult hides it in location B, the infant already has learned a general principle about this object's normal location. In an experiment, infants made the location error much more often if the adult performed the original hiding of the location A object with pedagogical social cues (such as eye contact and calling the child's name). The infants apparently believed adult instruction more than they believed their own eyes.

In the current study, Topál *et al.* report that pedagogical social cues affect domestic dogs in much the same way. Dogs also follow human instruction accompanied by such cues over their own visual experience. It should be noted, however, that the human also gave some pedagogical cues to the dog when hiding the object in location B. Why dogs nevertheless gave precedence to the information from the initial location A trials is still an open question.

Topál *et al.* also report that the manipulation of pedagogical cues does not affect human-raised wolves at all—they go with what they see in all cases. This finding provides strong support for the domestication hypothesis, by again showing striking dog-

wolf differences, and striking dog-human convergences—in this case, in a task with which most dogs have no previous experience.

But there is an interesting difference between dogs and human infants. Topál *et al.* observed that dogs did not make the location error if the person hiding the object in location B was not the same person who hid it in location A. Children made the error whether the person was the same or not. The authors interpret this as showing that human children are sensitive to true pedagogy—they, in essence, take instruction from all adults equally, considering it as general cultural information, whereas dogs are sensitive only to communication from humans about the immediate situation. It is possible that neither dogs nor any other nonhuman species communicate gener-

alized (normative) information in this way.

Dogs' special social-cognitive skills are not "normal" in that they do not gesture for or teach humans reciprocally, and they do not use their comprehension abilities with other dogs. They have evolved specialized skills for dealing with their unique situation in which they benefit by taking orders from humans. Indeed, a recent study has found more sophisticated communicative skills in dogs that have been directly selected by humans for specific tasks such as hunting and herding (12). Domestic dogs thus illustrate one way in which specialized cognitive skills may evolve to meet special ecological circumstances.

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10.1126/science.1179670

NEUROSCIENCE

Erasing Fear Memories

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Events that are associated with trauma and fear often leave memories that reoccur spontaneously, leading to excessive fear, anxiety, and, in some cases, posttraumatic stress disorder. Such relapses of fear memories constitute a major clinical problem, and their elimination is a major cornerstone of psychological therapy. Many neurobiological studies are therefore focused on understanding how fear memories are controlled (1). On page 1258 of this issue, Gogolla *et al.* (2) take an important step in the field by determining that the extracellular environment in a particular region of the brain—the amygdala—is responsible for making fear memories erasure-resistant.

"Extinction" is a popular behavioral technique to block recurring traumatic memories. This form of learning is characterized by a decrease in a fear response when the contingent relationship—between a conditioned stimulus (e.g., a sound) and an unconditioned stimulus (e.g., an electric shock)—is compromised. This situation is most commonly

implemented when the conditioned stimulus is repeatedly presented in the absence of the shock (3). It is now well accepted that extinction represents new learning and does not erase the preexisting memory (4). Indeed, the original memory can spontaneously recover, or it can be renewed, when the conditioned stimulus is presented in contexts different

Why are memories of traumatic events nearly impossible to eliminate?

from that in which the extinction protocol was administered. The resilience of traumatic memories to extinction represents a serious obstacle for treating disorders characterized by abnormal fear and anxiety.

Gogolla *et al.* were inspired by previous work originating from fields as diverse as development of fear conditioning (when fear is associated with a neutral stimulus) and plasticity of the visual cerebral cortex. These studies demonstrated that in contrast to the inability of an extinction protocol to erase the fear memory in adult rats, extinction of acquired fear in young rats (17 days after birth) deletes the fear memory (5). Further studies showed that sensitivity to erasure of fear memories is already lost at 23 days after birth. At all ages, extinction of fear conditioning in rats implicated neuronal circuits in the amygdala, a brain region necessary for fear memory acquisition and extinction. What changes

occur during amygdala development that are responsible for switching off the susceptibility of fear memory to the process of elimination?

Developmental windows during which neural plasticity is different from that of adult animals have been extensively studied in the mammalian visual cortex. Visual



Resisting erasure. Young mice as well as adults lacking chondroitin sulfate proteoglycans (CSPGs) in their amygdalae showed a faster extinction of the fear response (solid line) (which was acquired during prior fear conditioning), compared to adult mice treated with placebo (dashed line). When fear memory was retested 1 and 4 weeks after the extinction protocol, only the young mice and adults lacking CSPGs had completely eliminated the fear memory, whereas fear response had reoccurred in the placebo group.

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cortical circuits are particularly sensitive to visual deprivations during the so-called critical period. An important determinant of the closure of the critical period is maturation of the extracellular matrix that surrounds visual cortical neurons. Chondroitin sulfate proteoglycans (CSPGs) are the main constituents of the adult extracellular matrix. CSPGs condense into a netlike structure that surrounds a subclass of inhibitory interneurons (those that express the protein parvalbumin). This occurs in parallel with closure of the critical period. Eliminating CSPGs from the adult rat cortex (by injecting the enzyme chondroitinase ABC, which digests lateral chondroitin sulfate chains) can enhance plasticity almost to the level of a juvenile rat (6), suggesting that maturation of CSPGs endows the visual cortex with adultlike plasticity.

Like the visual cortex study, Gogolla *et al.* determined that maturation of the extracellular matrix in the amygdala is responsible for closing the developmental period during which fear memories are susceptible to extinction. The authors found that the number of CSPGs-containing perineuronal nets in the mouse amygdala increases sharply between 16 and 23 days after birth, in correlation with the developmental switch of fear memories from erasure-prone to erasure-resistant. Adult mice were injected in the amygdala with chondroitinase ABC to eliminate CSPGs, and then subjected to fear conditioning by pairing a sound with an electric shock.

Then, mice were subjected to an extinction protocol consisting of repeated presentation of the sound without the shock. To examine context specificity, the authors administered the extinction protocol in a context different from the one in which the animal had been previously conditioned. During the extinction protocol, the sound elicited progressively less fear response (cessation of body movement, or "freezing") both in mice treated with chondroitinase ABC and in control mice treated with placebo (saline solution). However, the rate at which the fear response decayed was much faster in chondroitinase ABC-treated mice than in control mice (see the figure). Strikingly, when response to the conditioned sound was retested 1 and 4 weeks after the extinction protocol, the fear response was not observed in animals treated with chondroitinase ABC—neither in the same context in which the extinction protocol had been administered, nor in the context in which the animals had originally been conditioned. This indicates complete loss of the fear memory. Thus, treating adult mice with chondroitinase ABC restores fear memory acquisition to the erasure-prone modality that typifies young rodents. By contrast, substantial spontaneous recovery and fear renewal occurred in control adult mice. Interestingly, chondroitinase ABC was effective if injected before fear conditioning, but not if injected before the extinction protocol. Thus, CSPGs are not directly regulating the processes occurring

during extinction, but they are important for coding an erasure-resistant memory during memory acquisition.

The results of Gogolla *et al.*, together with previous experiments on the visual cortex, suggest that maturation of the extracellular matrix could be a mechanism used by different brain circuits to change from a malleable to a more crystallized state during development. The presence of a high concentration of CSPGs in the perineuronal nets surrounding inhibitory neurons suggests that inhibitory circuits could play an important role in the developmental control of plasticity. Evidence supports this role for parvalbumin-expressing cells in the rodent visual cortex (7), but much less is known about other circuits. The mechanistic aspects of how CSPGs control plasticity are also still obscure: Is their organization in perineuronal nets important? What is the function of this structure at the molecular level? Further work is needed to understand how general this mechanism is and whether it applies to the human brain as well.

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10.1126/science.1179697

ECOLOGY

Threats to Freshwater Fish

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The sole wild population of the small live-bearing fish known as Picote de Tequila (*Zoogoneticus tequila*, see the figure) lives in a single, 4-m-wide pool in the Ameca Basin in Central Mexico. This pool supports a population of under 500 of these fish, of which fewer than 50 are adults (1). Trinidadian guppies (*Poecilia reticulata*) introduced to the area, possibly in an attempt to control mosquito larvae, outnumber them by 6 to 1 (1, 2). Exotic species are a well-known threat to freshwater fish populations, particularly those, like *Z. tequila*, that are vulnerable as a result of their reduced population size and low genetic diversity (3). Habitat

fragmentation, habitat loss, and overenthusiastic collecting further compound the risk of extinction for this and many other fish species. Biodiversity loss affects all taxa, but freshwater fish are especially susceptible because many fish species are rare. One way to help conserve rare species is to learn how naturally small and fragmented populations manage to persist in the wild.

Picote de Tequila is an exceptionally well-documented example of a fish on the brink of extinction, but the reasons it finds itself in this perilous state are depressingly familiar. Freshwater faunas worldwide are imperiled. Data for many regions are sparse or missing altogether, but the information that is available is chilling. A 2008 assessment found that some 40% of freshwater fish in continental North America are at risk or already lost

Insights into how small, isolated fish populations persist in the wild could aid conservation efforts.

(4). These 761 taxa (of which 230 are vulnerable, 190 threatened, 280 endangered, and 61 extinct or extinct in the wild) represent a 92% increase on the equivalent assessment in 1989 (4). Habitat degradation emerges as a substantial threat, as does the presence of non-native species. The same pattern is repeated in Europe (5–7).

There is no doubt that human activities have played a major role in the decline of freshwater fish populations, that the situation has deteriorated (4, 6, 8), and that prospects are poor in light of ever-increasing demands for water and natural resources and the projected impacts of climate change (9). The remedies—pollution control, restoration and improved management of freshwater habitats, limits on harvests of vulnerable species, and restrictions on the translocation of fish, par-

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ticularly exotic species—have been spelled out before (6). What is less appreciated is that the biological diversity of freshwater fish assemblages is as much a product of rarity as it is of richness. Understanding how small, isolated populations manage to persist in the wild in the absence of human disturbance could lead to new insights into the conservation of threatened species.

With an estimated 32,500 species, fish are the vertebrate group with by far the most species (10). Some 43% of these fish species are found in lakes, rivers, and other freshwater systems (10)—a remarkable figure given that freshwater habitats represent less than 0.01% of the water on Earth (11). Better resolution of taxonomic boundaries and more thorough exploration have resulted in a linear rise in the number of known fish species over recent decades and account for a small fraction of the increase in imperiled fish (4). Over 5000 species of freshwater fish have been discovered in the last 30 years alone (10).

This diversity is rooted in the habitat heterogeneity that freshwaters offer, and in the geographical isolation of different water bodies. There are many examples of radiations, and speciation can be rapid. For example, isolated populations of the Bahamas mosquitofish (*Gambusia hubbsia*) have speciated within the last ~10,000 years (12). Differentiation can occur even in the absence of physical barriers—for instance, between the bottom-dwelling and surface-dwelling forms of Arctic charr (*Salvelinus alpinus*) (13), or among populations of Malawi cichlids (*Pseudotropheus* spp.) on adjacent rocky headlands (14).

Freshwater habitats offer a range of ecological opportunities, and fish have proven themselves well able to exploit this potential. However, this diversification often occurs over small geographical scales and across fragmented landscapes. Many species therefore have a narrow range, are restricted to a few sites, or have small population sizes: They are intrinsically rare (15). Moreover, reproductive barriers between young species can be fragile. Learned mate recognition helps to maintain reproductive isolation among closely related cichlids in the Lake Victoria “species flock” (16); changes in water quality, such as eutrophication (17), that make it more difficult for members of a species to recognize one another lead to interbreeding and the blurring of species boundaries.

Although rarity is regarded as a risk factor for extinction, many rare fish species have



The perils of being naturally rare. Fish such as *Zoogoneticus tequila* may be naturally rare, but are now threatened through human-induced changes such as habitat loss and fragmentation.

persisted for long periods of time. Geographically isolated whitefish species—the pollan, powan, and vendace (*Coregonus* spp.)—have survived in the British Isles since the last ice age. Several splitfin species, such as *Characodon audax* and *Allotoca catarinae*, have naturally restricted distributions.

The idea that freshwater fish have evolved, as a by-product of rarity, mechanisms to resist extinction is intriguing. Adaptations such as sperm storage allow a single female guppy to found a viable population (18). Stable environments, such as those in caves or spring-fed lakes, could safeguard populations against demographic stochasticity. Isolated populations may be less affected by competition and predation, and protected against pathogens and parasites. Alternatively, the dendritic nature of a stream system or networks of ponds may provide colonists when small local populations go extinct (19). Polymorphisms, such as differentiation into bottom- and surface-dwelling forms, may reevolve if one variant is lost. Rare populations may also possess locally adapted genomes that have been purged of deleterious mutations (3).

Phylogenetically controlled tests are needed to determine whether some groups of freshwater fish are unusually able to tolerate rarity (20) and—assuming that this is not simply a matter of chance—which features of their evolutionary ecology help to maintain a viable population and nudge them away from extinction. What is not in doubt is that naturally rare species are at greater risk once humans are involved. European fish with small ranges are more threatened than closely related species with wider distributions (7). The same effect is seen in North American fish (4).

Setting aside some well-studied (often commercial) species such as salmon, rela-

tively little is known about the spatial and temporal dynamics of unperturbed freshwater fish populations and assemblages. Ecologists have recognized for decades that natural communities experience constant turnover, but the benchmarks used to set conservation targets tend to be static. Better awareness of the intrinsic changes in these systems, particularly in relation to rare species, will help to delineate achievable conservation outcomes (including the appreciation that some taxa will become extinct).

Rarity itself deserves more study. Are the different ways of being uncommon (15) equally pervasive, and do they have different implications for persistence, particularly in the face of anthropogenic change? The genomics of rarity are also only just beginning to emerge. Clearly, the best chance of conserving the “tequila” fish will be to restore its habitat and allow the population to increase (3). But whether the reintroduction of conspecifics from zoo populations would help secure the future of the species through the infusion of greater genetic diversity, or hinder it by disrupting the genotype and contributing traits evolved in response to domestication, remains an open question.

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21. I acknowledge the Royal Society of Edinburgh for a Support Fellowship.

Conserved Functions of Membrane Active GTPases in Coated Vesicle Formation

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Coated vesicles concentrate and package cargo molecules to mediate their efficient transport between intracellular compartments. Cytosolic coat proteins such as clathrin and adaptor complexes and coat protein complex I (COPI) and COPII self-assemble to deform the membrane and interact directly with cargo molecules to capture them in nascent buds. The guanine triphosphatases (GTPases) Arf, Sar1, and dynamin are core components of the coated vesicle machinery. These GTPases, which associate with and dissociate from donor membranes in a guanine triphosphate-dependent manner, can also actively remodel membranes. Recent evidence suggests that, although structurally diverse, Arf family GTPases and dynamin may play mechanistically similar roles as fidelity monitors that govern cargo packaging and coated vesicle maturation and as components of the fission machinery to mediate vesicle release.

The modular organization of the eukaryotic cytoplasm into membrane-bound organelles contributes to increased efficiency and specificity of chemical reactions. Organelles communicate with each other through vesicular transport, where information is packaged and transported in small vesicles. Cargo routing and speed of delivery are thus key determinants for successful information transfer between compartments.

Coated vesicular transport is a specialized form of cargo transfer involving vesicles enveloped by a discernable coat. Coated vesicles achieve high selectivity as protein cargo transporters because of their high protein-to-lipid ratio (*1*). Clathrin-coated vesicles (CCVs) mediate transport between the plasma membrane, endosome, and trans Golgi compartments, whereas coat protein complex I (COPI)– and COPII-coated vesicles mediate transport from the Golgi and endoplasmic reticulum (ER), respectively (*2–4*). Coat complexes perform the dual role of concentrating cargo and sculpting membranes in the process of generating nascent transport vesicles. Growing evidence points to a role for membrane-active guanine triphosphatases (GTPases) in monitoring the fidelity and efficiency of these processes. Arf1 [adenosine diphosphate (ADP) ribosylation factor 1] functions in the formation of COPI vesicles and trans Golgi-derived CCVs, Sar1 (secretion-associated RAS-related protein 1) in COPII vesicle formation at the ER, and dynamin in CCV formation at the plasma membrane. Recent studies indicate that these GTPases show important mechanistic similarities in terms of their role in cargo packaging and their ability to actively remodel membranes. Here, we review these findings and propose gen-

eral models that might explain how these GTPases regulate the formation of coated vesicles.

First Principles of Coated Vesicle Formation

Coated vesicle formation can be viewed as a progression of three stages: (i) assembly of coat subunits on the membrane, during which cargo recognition and sorting take place; (ii) maturation, during which the coat and underlying membrane acquire curvature; and (iii) scission to release a coated vesicle (*5, 6*) (Fig. 1). Coat assembly and maturation, although conceptually distinct, are in fact intricately linked and temporally overlapping stages that are required for efficient cargo capture and packaging. The efficiency of coated vesicle-

mediated transport is determined both by their ability to select and concentrate cargo molecules during assembly and maturation and by the overall rate of the maturation process and membrane scission leading to vesicle formation.

The three major classes of coated vesicles (CCV, COPI, and COPII) are distinct in their protein and lipid composition and recognize distinct sets of cargo. Two cytosolic protein complexes, clathrin triskelia and heterotetrameric adaptor proteins for CCVs and the Sec13-31 and Sec23-24 complexes for COPII vesicles, assemble on the membrane to form the coat. A single, multisubunit coatomer complex assembles to form the COPI coat. In spite of these compositional differences, coated vesicles show architectural similarities. For example, each coat consists of an outer cage composed of polymerized protein complexes that contain N-terminal β -propeller-WD40 and C-terminal α -solenoid motifs (*7, 8*). Biochemical and structural studies have shown that these protein complexes exhibit a tendency to self-assemble into empty spherical cages (*9–11*). Cage subunits per se do not bind membranes and need to be recruited by an inner adaptor layer. The adaptor proteins display remarkable diversity in their structural characteristics but are similar in their capacity for simultaneous recognition of multiple membrane-bound signals such as sorting motifs in cargo, additional coat proteins, and negatively charged membrane lipids. Even in solution, adaptors promote self-assembly of the outer cage layer subunits and modulate their sizes by recruiting more cage subunits and/or by structurally affecting the inherent flexibility of cages (*12, 13*).

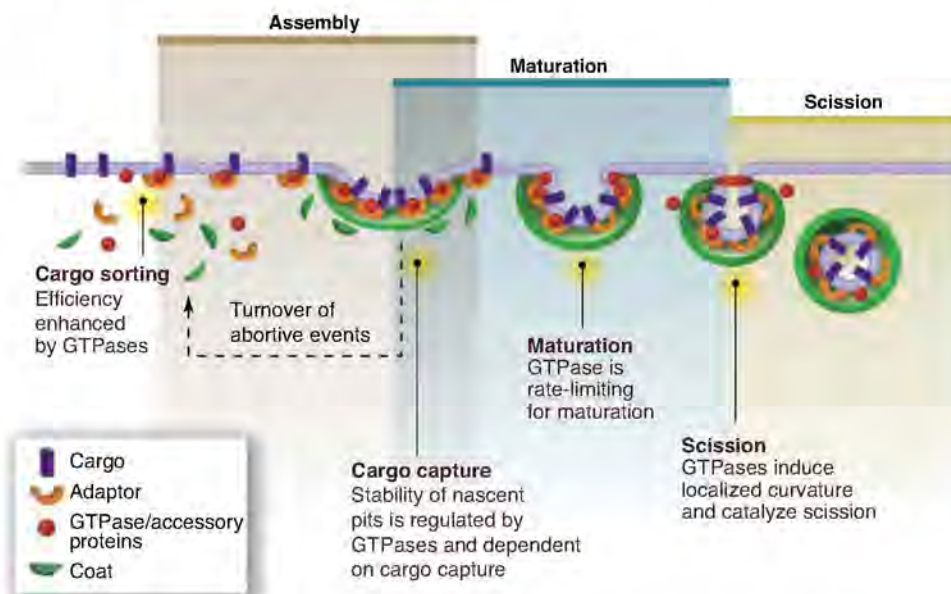


Fig. 1. General scheme of coated vesicle formation. This process involves the initial assembly of coat subunits on the membrane, during which cargo recognition and sorting takes place, followed by maturation, during which the coat and underlying membrane acquire curvature. Scission of the coated bud finally generates a coated vesicle. The reported involvement of the membrane active GTPases, Arf1, Sar1, and dynamin, during each of these stages is described.

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The membrane acts as a platform to concentrate coat proteins and imparts considerable resistance to bending during formation of coated vesicles (14). Coats recruit additional proteins to counter this resistance. COPI and COPII coated vesicles display a functional conservation in the coat components such that members of the Arf family of GTPases appear to manage both coat recruitment and curvature generation in membranes (8). In addition to the GTPase dynamin, plasma membrane-derived CCVs recruit accessory proteins such as amphiphysin, epsin, endophilin, and/or members of the sorting nexin family, which have all been shown to bind the core coat components and to generate and/or recognize membrane curvature (8, 15). The accessory proteins interact with the membrane through structural elements such as BAR (Bin, amphiphysin, Rvs) and ENTH (epsin N-terminal homology) domains (16). Unlike the GTPases (see below), membrane binding and/or dissociation of these proteins is not known to be induced or triggered, and thus they show a "passive" mode of membrane association. Although they may not be needed to generate curvature per se, they might play a role in stabilizing or locking in the coat-generated curvature and/or in fine-tuning the rate and degree of curvature generation to enhance the efficiency of cargo loading and the ability to accommodate structurally diverse cargos. The GTPases, on the other hand, associate with membranes through a more "active" switch-type mechanism dependent on GTP binding and/or hydrolysis. Such switching might control the generation of membrane curvature during coat maturation and/or trigger scission. The importance of this feature is conveyed by the fact that their involvement is critical to all forms of coated vesicular transport.

Arf1 in COPI vesicle formation. Arf family GTPases constitute members of the Ras superfamily (17) that display low rates of guanosine diphosphate (GDP) dissociation and GTP binding and thus require extrinsic GTP exchange factors (GEFs). In addition, they have an intrinsically low rate of GTP hydrolysis that is dramatically stimulated by extrinsic GTPase activating proteins (GAPs). The GTP-bound state is often associated with biological activity, such as binding to effectors, whereas the inactive, GDP-bound state does not bind effectors. Externally provided GEF and GAP activities are required to transition the GTPases between GTP-bound, active and GDP-bound, inactive states.

Arf1 initiates COPI coated vesicle formation by recruiting the coatamer complex to the membrane (18). Arf1 associates with the membrane in a GTP-dependent manner, via a short amphipathic N-terminal helix that is covalently modified with a myristoyl chain (Fig. 2). In its GDP-bound

N-terminal helix would cause an asymmetric expansion of the outer versus the inner leaflet of the lipid bilayer, thereby causing it to bend. Recent evidence also indicates that GTP-bound, membrane-active Arf1 can form dimers, which could stabilize regions of high membrane curvature (19).

Growing evidence points to an intricate relationship between GEF- and GAP-mediated GTP binding and hydrolysis by Arf1 and membrane curvature. GBF-1 (Golgi-specific brefeldin A-resistance factor 1) and ArfGAP1, which are the Golgi-localized GEF and GAP for Arf1, are peripherally associated membrane proteins involved in COPI vesicle formation (17). GBF-1 is thought to associate with Golgi membranes through interactions with resident membrane proteins (22). GTP binding promoted by GBF-1 causes Arf1 to associate with membranes, which could result in the generation of membrane curvature. ArfGAP1, on the other hand, binds membranes through a motif termed ArfGAP1-like lipid packing sensor (ALPS). ALPS motifs are amphipathic helices lined by residues that pack favorably into membranes of high curvature (23). Thus, ArfGAP1 displays higher affinity for and is activated by curved membranes, thereby selectively promoting GTP hydrolysis by Arf1 on curved membranes.

Sar1 in COPII vesicle formation. Like Arf, Sar1 is a Ras-related GTPase that initiates COPII coated vesicle formation by recruiting the adaptor complex Sec23-24 to the membrane (18). Sar1 also shows similarities to Arf1 in terms of GTP binding and hydrolysis-dependent membrane association properties. GTP loading of Sar1 exposes a long N-terminal amphipathic helix, which is not myristoylated, but nonetheless mediates membrane binding (Fig. 2). Binding of Sar1 to liposomes induces formation of membrane tubules with an outer diameter of ~26 nm (Fig. 2) (24, 25). Neither Sar1 mutants deleted in residues that compose the amphipathic helix nor

wild-type GDP-bound Sar1 can associate with membranes. The GEF for Sar1 is Sec12, an integral ER-localized membrane protein. The COPII adaptor protein Sec23-24 and outer coat protein Sec13-31 both function as GAPs causing GTP hydrolysis on Sar1 (26).

Dynamin Family of GTPases in CCV Formation

The dynamin family of GTPases show biochemical properties that are distinct from those of the small Ras family GTPases (27). They display a low affinity for GTP and high rates of GDP dissociation. Nucleotide exchange can thus occur

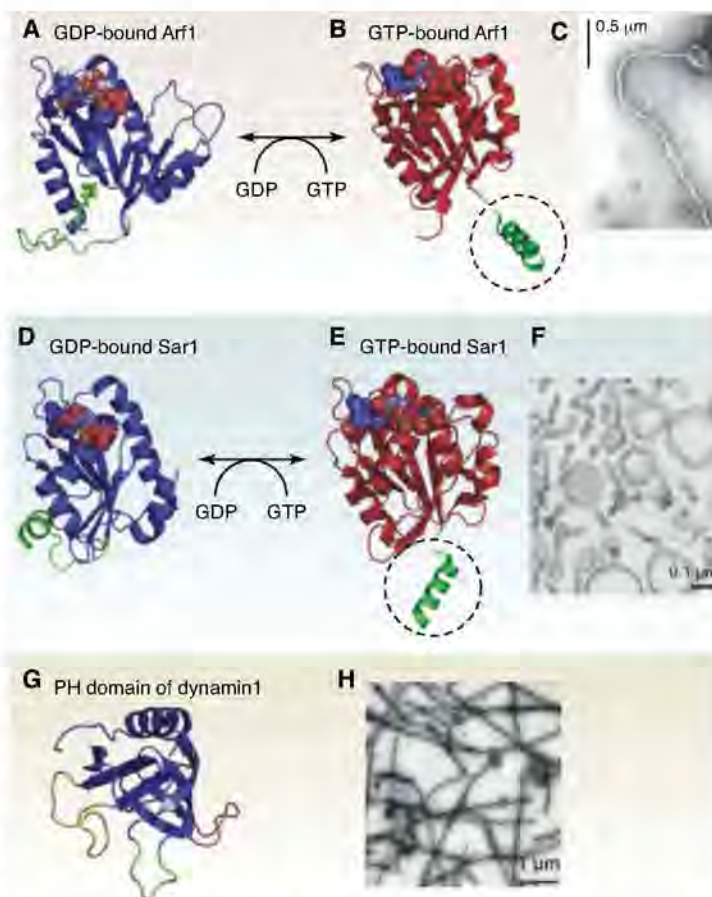


Fig. 2. Membrane active GTPases in coated vesicular transport. Structures were rendered by using PyMol (www.pymol.org). (A) Structure of myristoylated Arf1 in the GDP-bound state [Protein Data Bank (PDB) accession code 2K5U] (52). The myristoyl chain and the first 16 residues are shown in green. (B) Structure of Arf1 in the GTP-bound state (PDB 1O3Y) (52). Gly¹⁶ and a schematic representation of the exposed N-terminal helix without the myristoyl chain (dotted circle) are shown in green. (C) Membrane tubules formed by GTP-bound Arf1 (20). (D) Structure of GDP-bound Sar1 (PDB 2GAO). Residues 13 to 26 are shown in green. (E) Structure of GTP-bound Sar1 (PDB 1M2O) (53). Gly²⁴ and a representation of the extended N-terminal helix (dotted circle) are shown in green. (F) Membrane tubules formed by GTP-bound Sar1 (24). (G) Structure of dynamin 1 PH domain (PDB 1DYN) (54). The variable loops VL1 (green), VL2 (red), and VL3 (gold) are highlighted. (H) Membrane tubules formed by dynamin 1.

state, the helix is buried in the molecule, but GTP binding triggers a conformational change in the molecule that exposes the amphipathic helix. As a result, Arf1 has a higher affinity for the membrane in the GTP bound state. Recent results show that, upon binding, Arf1 can remodel membranes into highly curved tubules with an outer diameter of ~45 nm (Fig. 2) (19, 20). GDP-bound Arf1 or Arf mutants with polar substitutions in the amphipathic helix do not bind membranes and, as a result, are not membrane active. Curvature generation by Arf1 can be explained by the bilayer-couple model (21), where the insertion of the amphipathic

spontaneously without external GEFs. Dynamin family members also show high basal rates of GTP hydrolysis, which is further stimulated upon self-assembly. Dynamin 1, the prototypical member of this family, is involved in CCV formation from the presynaptic membrane, whereas dynamin 2 functions in clathrin-mediated endocytosis in nonneuronal cells (28).

Dynamin is a multidomain GTPase that binds membranes via a pleckstrin homology (PH) domain, which provides docking sites for negatively charged lipid headgroups and contains three loops, the first of which is lined by hydrophobic residues (Fig. 2). Dynamin interacts with multiple Src homology 3 (SH3) domain-containing endocytic accessory proteins through its C-terminal proline-arginine-rich domain (PRD). Despite its ability to bind directly to membranes *in vitro*, *in vivo* studies have shown that PRD-SH3 domain protein interactions are required to recruit dynamin to clathrin-coated pits (29). Dynamin has a high propensity to self-assemble under low ionic strength conditions or under physiological conditions on a membrane template. Self-assembly considerably stimulates basal GTP hydrolysis, in part mediated by an intramolecular GTPase effector domain (GED) (30, 31). Although the mechanism remains unknown, self-assembly activates an intrinsic GAP activity in dynamin. Despite its ability to bind liposomes in a nucleotide-independent manner, recent results using site-specific labeling of residues in the PH domain with environment-sensitive fluorescent probes suggest that GTP binding and hydrolysis by dynamin 1 provide a reversible switch for membrane association (32). Thus, dynamin 1 shows higher membrane binding in the apo or GTP-bound state and lower binding in the GDP-bound state. When analyzed in the presence of GTP, dynamin 1 binds first and then dissociates from the membrane, reflecting a cycle of association, spontaneous self-assembly, GTP hydrolysis, and dissociation. Residues in the PH domain show significant insertion into the lipid bilayer, and this, together with dynamin's scaffolding activity, could induce and stabilize membrane curvature (32, 33). Indeed, membrane binding of dynamin causes the formation of membrane tubules with an outer diameter of ~45 nm (Fig. 2) (34).

GTPases regulate cargo sorting during coated vesicle formation. Proofreading mechanisms ensure sorting of bona fide cargo and maximize cargo uptake, thereby contributing to the fidelity and efficiency of vesicular transport. Coat adaptors display multiple independent binding sites for cargo, coat components, and negatively charged lipids. Because binding affinities for polyvalent interactions are much higher compared with those of monovalent interactions, coincident detection of such binding partners contributes to the spatiotemporal coordination of coat assembly. Thus, coat assembly occurs on the right cellular compartment and when adaptors engage with as many partners as possible.

Several reports suggest the involvement of GTPases in regulating cargo sorting during coated

vesicular transport (Fig. 1). GTP-bound, membrane-anchored Arf1 interacts with cargo-ArfGAP1 and cargo-coatamer β subunit complexes, whereas GTP-bound Sar1 interacts with cargo-Sec23-24 complexes to couple cargo recruitment to coat assembly (35, 36). This form of cargo delivery in complex with coat components ensures efficient incorporation of essential cargo such as SNARE (soluble *N*-ethylmaleimide-sensitive factor attachment protein receptor) molecules into coated vesicles. The involvement of Arf family GTPases in these early stages provides an avenue for regulation of cargo sorting into coated pits via a kinetic proofreading mechanism based on the stability of the cargo, GTPase, and coat complex (Fig. 1). Thus, given the intrinsic Sar1 GAP activity of the COPII coat, Sar1 and Sec23-24 complexes interacting with bona fide ER-associated cargo molecules will be longer-lived than those not interacting with cargo molecules on the membrane (37). Similarly, ArfGAP1 activity can be regulated by cargo and coat interactions, and in this way coat assembly can be tightly coupled to efficient cargo recruitment (38). In the case of plasma membrane-derived CCVs, dynamin may also indirectly control the efficiency of cargo sorting but by a different mechanism (Fig. 1). Recent results using total internal reflection fluorescence microscopy (TIR-FM) to monitor the dynamics of clathrin coats on the plasma membrane combined with statistical analyses indicate that dynamin controls early decision-

making and rate-limiting events in CCV formation (6). Thus, reducing dynamin concentrations in cells decreases the rate of turnover of early abortive species and increases the maturation time of coated pits, whereas expression of dynamin point mutants impaired in self-assembly leads to faster turnover of abortive species and increased endocytic uptake of cargo (30). These results have been rationalized on the basis of a model in which dynamin acts as a fidelity sensor that monitors maturation efficiencies of coated vesicles. Live-cell microscopy has also demonstrated that specific cargo molecules (e.g., heterotrimeric GTP-binding protein-coupled receptors) can regulate dynamin recruitment to CCPs and thus regulate the kinetics and fidelity of endocytic sorting events (39).

Membrane Fission During Coated Vesicular Transport

Release of coated vesicles requires the membrane necks of coated buds to undergo fission, a process requiring GTP hydrolysis. This is apparent from early studies where attached membrane buds arrested before the late step of scission accumulated in cells or synaptosomes perfused with non-hydrolyzable GTP analogs (40, 41). Reconstitution studies using liposomes have identified the minimal components necessary for the formation of COPI and COPII vesicles (42, 43). Early studies in these minimal systems indicated that GTP-

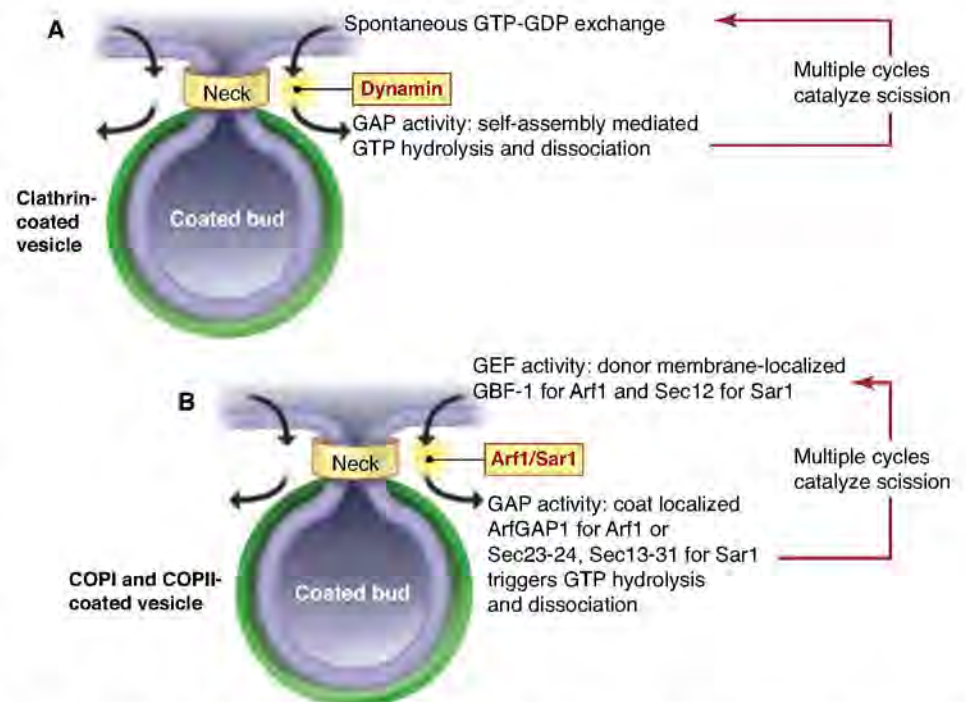


Fig. 3. General model for scission of coated buds. We propose that the restricted localization of membrane active GTPases to the necks of budding coated vesicles could catalyze membrane fission. In the case of clathrin-coated buds (A), dynamin is localized at the neck where multiple cycles of membrane association, spontaneous self-assembly, and GTP hydrolysis produce the GDP-bound state, and membrane dissociation leads to scission. The donor membrane-localized GEF activity and coat-localized GAP activity could restrict Arf1 and Sar1 localization to the necks of COPI- and COPII-coated buds (B). As a consequence, reversible membrane association of these GTPases at the neck could eventually lead to scission of the coated bud.

bound Arf1 and Sar1 could both recruit coat proteins to the membrane and cause scission. However, more recent studies that monitored release of coated vesicles under milder conditions, which avoided shearing of budded membranes, suggest that GTP hydrolysis is indeed necessary for coat scission (25, 44). Similar reconstitution studies have not been performed to identify the minimum components necessary for CCV formation, although clathrin-mediated endocytosis in perforated cells is blocked at a late stage by non-hydrolyzable GTP analogs (45).

Dynamin 1-mediated membrane fission has been reconstituted on liposomes and has been shown to require GTP hydrolysis (34, 46). More recently, dynamin-catalyzed membrane fission has been studied in greater detail by using a system of supported bilayers with excess reservoir or SUPER templates (47) and by direct conductivity measurements of membrane tubules (48). Results from these studies could provide a general model for coated vesicle scission catalyzed by membrane active GTPases (Fig. 3). Previous models suggested that dynamin-mediated fission required a power stroke generated by a concerted conformational change in assembled dynamin and triggered by rapid GTP hydrolysis (27, 34, 46, 49). However, more recent studies have shown that dynamin assemblies stabilize highly curved templates (48) and that membrane fission requires GTP hydrolysis-dependent reversible membrane association of dynamin (47). During these cycles, and because of dynamin's ability to induce curvature, the underlying membrane undergoes squeezing and relaxation, resulting in the stochastic generation of a hemifission intermediate and thereby causing fission (47, 48). Thus, a seemingly futile cycle of membrane binding and GTP hydrolysis-dependent dissociation is in fact necessary for dynamin-catalyzed membrane fission.

It is tempting to speculate that Arf1 and Sar1 could function similarly during scission of COPI and COPII vesicles (Fig. 3). Dynamin is self-sufficient in catalyzing membrane fission because it does not require an external GEF or GAP for GTP hydrolysis and reversible membrane association. At the necks of budded COPI and COPII vesicles, both Arf1 and Sar1 could acquire properties similar to dynamin 1. At late stages of vesicle formation, the GAPs for Arf1 and Sar1, that is, the curvature-sensitive ArfGAP and coat components Sec23-24 and Sec13-31, are present exclusively on the membrane bud, whereas the GEFs, GBF-1, and Sec12 are restricted to the more planar donor membrane. Moreover, recent studies showed that lipid-packing defects associated with regions of high membrane curvature could promote spontaneous insertion of Arf1's amphipathic helix, thereby allowing GDP-GTP exchange (50). In principle, this effect could obviate the need for

GEF activity at the necks of budded vesicles. Thus, due in part to the spatial segregation of GEFs and GAPs and to their local high curvature, the necks of budded vesicles could represent a region where the combined influence of GEFs and GAPs promote GTP binding and hydrolysis, thereby causing Arf1 and Sar1 to undergo multiple cycles of membrane binding and release (Fig. 3). This dynamic behavior would mirror that of dynamin 1 and could thus catalyze scission and coated vesicle release by a common mechanism.

Future Perspectives

Although we have a good knowledge of what makes a coated vesicle (i.e., its constituents), we still have little understanding of how a coated vesicle is made (i.e., the mechanisms governing vesicle formation). Better assays are needed to reconstitute the transport of cargo in a biochemically defined environment. Fluorescence-based approaches to monitor protein-protein and protein-lipid interactions along with assays that distinguish stages of budding and fission would provide the much-needed dynamic information. Most of our understanding of vesicular transport is derived from the read-outs of bulk assays. Bulk read-outs may be insufficient to understand cargo sorting during coated vesicular transport because cargo transport is a function of both cargo density per vesicle and the number of vesicles. Results from such assays average out possible differences between cargo content among individual vesicles. It is thus important to interrogate the process of coated vesicle formation at the single vesicle level. TIR-FM-based analysis is easily applied to cell surface phenomena such as clathrin-mediated endocytosis but may not be a viable option for intracellular vesicular transport. However, such analysis in reconstituted systems should provide a clearer understanding of the process of cargo sorting and vesicle formation. We may find interesting mechanistic differences between the different classes of coat proteins, but a deeper understanding may also reveal more mechanistic similarities than currently appreciated.

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55. We thank members of the Schmid lab, A. Razvi, and S. Kalipatnapu for critical comments on the manuscript. We apologize to those authors whose work we could not cite because of space constraints. This work was supported by a career development grant from the Leukemia and Lymphoma Society to T.J.P. and NIH grants (GM42455, MH61345, and GM73165) to S.L.S. This is TSRI manuscript number 20182.

10.1126/science.1171004

Shor's Quantum Factoring Algorithm on a Photonic Chip

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The realization of a quantum computer presents an exciting prospect of modern science. The processing of information encoded in quantum systems admitting quantum superposition and entanglement enables exponentially greater power for particular tasks. Originally conceived in the context of simulating complex quantum systems, it was the development of Shor's quantum factoring algorithm (1) that showed the capability of factoring the product of two large prime numbers exponentially faster than any known conventional method (2), which has ignited efforts to fabricate such a device.

Despite progress toward this goal, proof-of-principle demonstrations of Shor's algorithm have so far only been possible with liquid-state nuclear magnetic resonance (3) and bulk optical implementations of simplified logic gates (4, 5), owing to the need for several logic gates operating on several qubits, even for small-scale compiled versions. However, these approaches cannot be scaled to a large number of qubits because of purity, size, and stability limitations of these systems. We demonstrate a compiled version of Shor's algorithm operating on four qubits in which the processing occurs in a photonic circuit of several one- and two-qubit gates fabricated from integrated optical waveguides on a silica-on-silicon chip (6, 7). Whereas the full

Shor's algorithm is designed to factorize any given input, a compiled version is designed to find the prime factors of a specific input.

The quantum circuit our device implements is the compiled version of Shor's algorithm for factorizing 15 (3–5) (Fig. 1A). This algorithm uses five qubits, one of which, x_0 , is effectively redundant because it remains in a separable state throughout. The physical implementation (Fig. 1B) consists of two nondeterministic controlled-phase (CZ) gates (each with success $P = 1/9$, conditional on post selection) and six one-qubit Hadamard (H) gates (8). The computation proceeds as follows (Fig. 1, A and B): Four photons are input into the "0" or "1" waveguides to prepare the initial state $|\psi_{in}\rangle = |0\rangle_{x_0}|0\rangle_{x_1}|0\rangle_{f_1}|1\rangle_{f_2}$ (this does not represent 15 but rather the initialization for the compiled algorithm to compute the factors of 15). The H gates, implemented by 1/2 reflectivity directional couplers, then prepare each qubit in a superposition of 0 and 1, such that the entire state is a superposition of all possible four-bit inputs—part of the massive parallelism that gives rise to quantum speed-up. The core process is then performed by two independent CZ gates, each implemented by a network of three 1/3 directional couplers, that create a highly entangled output state (4, 5). Measurement of the output state of qubits x_1 and x_2 and classical processing give the results of the computation (9).

We simultaneously prepared four 790-nm photons via parametric down conversion, coupled them into and out of the chip with butt-coupled arrays of optical fibers, and detected them with silicon avalanche photo diodes at a typical coincidental rate of 100 Hz per measurement (integrated for 30 s). We input the state $|\psi_{in}\rangle$ and measured the output state of qubits x_1 and x_2 ; the output statistics (Fig. 1C) show the four binary outcomes: 000, 010, 100, and 110 (including the x_0 qubit). Outputs 010 and 110 lead to the correct calculation for finding the order $r = 4$ for the algorithm (9), which then enables efficient classical computation of the factors 3 and 5; 100 gives the trivial factors (1 and 15); and 000 is an expected failure mode inherent to Shor's algorithm. The measured results have a fidelity of $99 \pm 1\%$ with the ideal probability distribution (dashed line).

This demonstration of a small-scale compiled Shor's algorithm on a chip shows promise for quantum computing in integrated waveguides. Although it currently uses an inefficient single photon source and modest efficiency detectors, ongoing progress to address heralded gates and efficient sources and detectors (8) combined with the results presented here will allow large-scale quantum circuits on many qubits to be implemented. Any quantum computer is a many-particle, many-path interferometer; the capability to implement such complex interferometers in a stable and miniaturized architecture is therefore critical to the future realization of large-scale quantum algorithms.

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10. We thank R. Jozsa, A. Laing, A. Montanaro, S. Takeuchi, M. G. Thompson, and X.-Q. Zhou for helpful discussions. This work was supported by Engineering and Physical Sciences Research Council, Quantum Information Processing Interdisciplinary Research Collaboration, Intelligence Advanced Research Projects Activity, and the Leverhulme Trust. J.L.O.B. acknowledges a Royal Society Wolfson Merit Award.

Supporting Online Material

www.sciencemag.org/cgi/content/full/325/5945/1221/DC1
Materials and Methods
Fig. S1

18 March 2009; accepted 1 July 2009
10.1126/science.1173731

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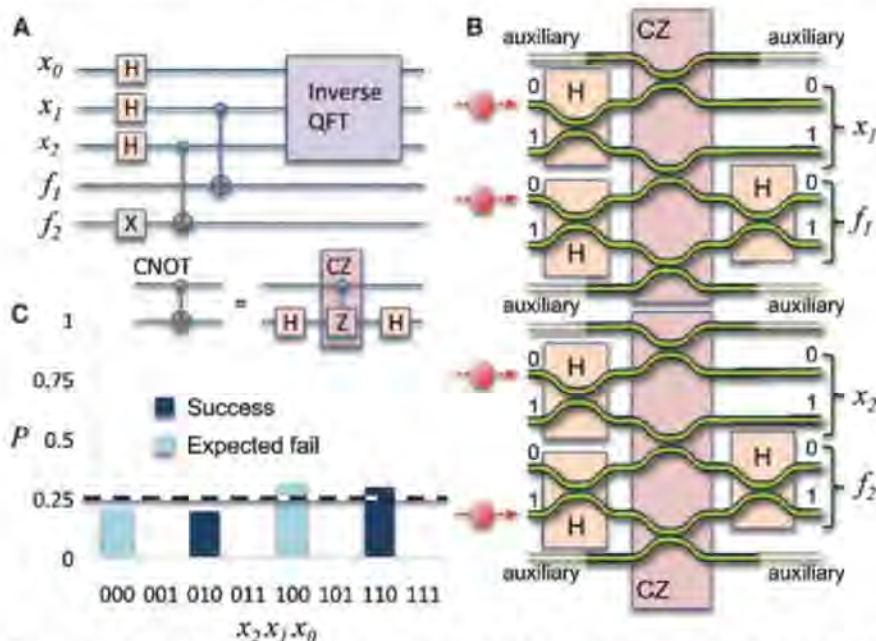


Fig. 1. Integrated optical implementation of Shor's quantum factoring algorithm. (A) The quantum circuit. QFT indicates quantum Fourier transform (9); CNOT, two qubit controlled NOT. (B) Schematic of the waveguide on chip device that implements the quantum computation. The x_n qubits carry the result of the algorithm; f_n are additional qubits required for the computation to work. (C) Outcomes of the algorithm.

An Ultramassive, Fast-Spinning White Dwarf in a Peculiar Binary System

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White dwarfs typically have masses in a narrow range centered at about 0.6 solar mass ($M_{\odot}$). Only a few ultramassive white dwarfs (mass $> 1.2 M_{\odot}$) are known. Those in binary systems are of particular interest, because a small amount of accreted mass could drive them above the Chandrasekhar limit, beyond which they become gravitationally unstable. Using data from the x-ray multimirror mission (XMM)–Newton satellite, we show that the x-ray pulsator RX J0648.0–4418 is a white dwarf with mass $> 1.2 M_{\odot}$, based on dynamical measurements only. This ultramassive white dwarf in a post-common envelope binary with a hot subdwarf can reach the Chandrasekhar limit, and possibly explode as a type Ia supernova, when its helium-rich companion will transfer mass at an increased rate through Roche lobe overflow.

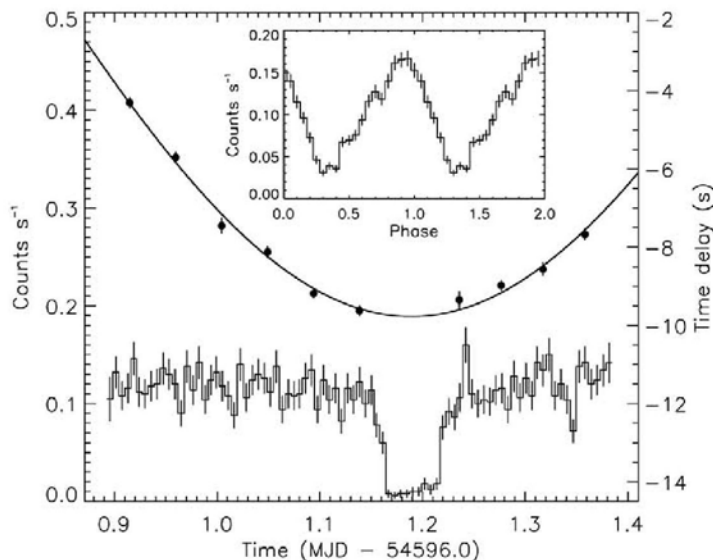
The only known binary system composed of an early type subdwarf star (the stripped core of an intermediate-mass star that lost its hydrogen outer layers during nonconservative mass transfer) and a pulsating x-ray source is HD 49798/RX J0648.0–4418. Its optical/ultraviolet (UV) emission is dominated by the subdwarf HD 49798, which, being very bright (V-band magnitude of 8.3, spectral type sdO5.5), has been extensively studied. The orbital period [$P_{\text{orb}} = 1.5476637(32)$ days], the projected semi-major axis [$A_C \sin(i) = (2.506 \pm 0.014) \times 10^{11}$ cm, where i is the inclination angle of the binary orbit], and the optical mass function [$f_{\text{opt}} = 4\pi^2/(GP_{\text{orb}}^2)[A_C \sin(i)]^3 = 0.263 \pm 0.004 M_{\odot}$, where G is the gravitational constant] of HD 49798 have been accurately determined (1–3), but the nature of the companion star remained completely unknown until periodic x-ray pulsations at $P = 13.2$ s were discovered in 1996 (4). This discovery demonstrated that the companion star was either a neutron star or a white dwarf. However, because its x-ray luminosity is much smaller than that expected from a neutron star accreting in the stellar wind of HD 49798 (5), it has been concluded that RX J0648.0–4418 is a white dwarf (6). The process that led to the formation of this peculiar system is still poorly understood.

The presence of fast x-ray pulsations makes the system equivalent to a double-lined spectroscopic binary, for which all the parameters and, in particular, the masses of the two components can be derived. With this objective, we observed HD 49798/RX J0648.0–4418 for 44×10^3 s on 10 to

11 May, 2008, with the Newton x-ray Multimirror Mission (XMM-Newton) satellite. The data were obtained with the European Photon Imaging Camera (EPIC), which provides imaging with high sensitivity and medium-resolution spectroscopy in the 0.15- to 12-keV energy range (7, 8). Based on the optical ephemeris (3), the observation was scheduled to include the orbital phase at which the x-ray eclipse could be expected ($\Phi = 0.75$). Previous x-ray observations (4, 9) did not cover this orbital phase. Our x-ray light curve (Fig. 1) shows the presence of an eclipse lasting ~ 1.3 hours, which allowed us to refine the measurement of the orbital period (see legend of Fig. 1) and derive the system's inclination.

Fig. 1. X-ray light curve of RX J0648.0–4418 in the 0.15- to 0.4-keV energy range obtained with the EPIC instrument on the XMM-Newton satellite. According to the optical ephemeris (3), the eclipse center was predicted to occur at modified Julian day (MJD) = $54,597.169 \pm 0.023$. The small difference with respect to the observed value (MJD = $54,597.189 \pm 0.015$) allowed us to determine the orbital period $P_{\text{orb}} = 1.5476666 \pm 0.0000022$ days, which is consistent with, but more precise than, the previous value

$P_{\text{orb}} = 1.5476637 \pm 0.0000032$ days (3). The points indicate the time delays of the x-ray pulsations measured at different orbital phases. They are induced by the orbital motion of the x-ray source and are well fitted by a sinusoidal curve with period fixed at P_{orb} and amplitude $A_X \sin(i) = 9.78 \pm 0.06$ light-s. (Inset) Pulse profile in the 0.15- to 0.4-keV energy range repeated for clarity over two cycles. The spin period $P = 13.18425 \pm 0.00004$ s has been determined with a standard phase fitting procedure.



The x-ray pulsations from RX J0648.0–4418 were detected in the EPIC data, with a broad single pulse and a pulsed fraction of 62% in the 0.15- to 0.4-keV energy range (Fig. 1, inset). By cross-correlating the folded light curves obtained at 10 different orbital phases with an average template, we determined the delays in the times of arrival of the pulses that were induced by the orbital motion (Fig. 1). By fitting them with a sinusoid [optical/UV spectroscopy indicates that the orbit is circular (1, 3)], we derived the projected semi-major axis $A_X \sin(i) = 9.78 \pm 0.06$ light-s. This corresponds to an x-ray mass function $f_X = 4\pi^2/(GP_{\text{orb}}^2)[A_X \sin(i)]^3 = 0.419 \pm 0.008 M_{\odot}$ that, combined with the optical mass function, yields the masses of the two stars as a function of the orbital plane inclination (Fig. 2). The inclination can be constrained by the duration of the x-ray eclipse, because it is related to the radius R_C of HD 49798 by $(R_C/a)^2 = \cos^2(i) + \sin^2(i) \sin^2(\Theta)$ (where a is the orbital separation and Θ is the eclipse half angle). The value $R_C = 1.45 \pm 0.25$ solar radii ($R_{\odot}$) derived for HD 49798 through optical spectroscopy (2) implies an inclination in the range 79° to 84° (Fig. 2). The corresponding masses are $M_X = 1.28 \pm 0.05 M_{\odot}$ for the white dwarf and $M_C = 1.50 \pm 0.05 M_{\odot}$ for HD 49798. Independently of the dimensions of HD 49798, a firm lower limit of $1.2 M_{\odot}$ (2σ confidence level) on the white dwarf mass is obtained for the extreme case $i = 90^\circ$, making RX J0648.0–4418 one of the most massive white dwarfs currently known (10–14).

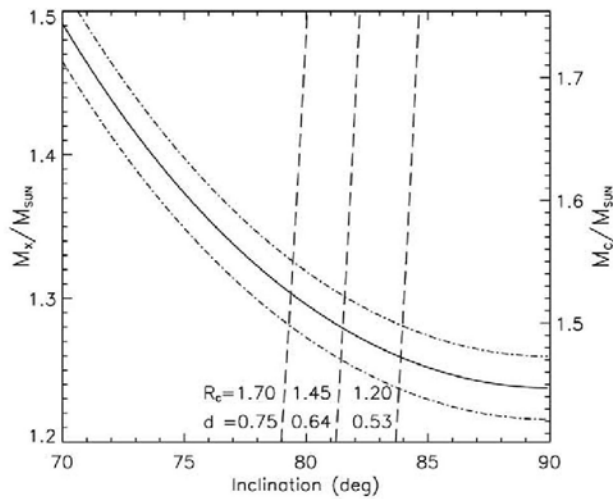
This peculiar system is thought to be the outcome of a common envelope evolution. HD

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Fig. 2. Masses of the HD 49798 subdwarf (right axis) and of its white dwarf companion (left axis) as a function of the orbital plane inclination. The solid line gives the values (and 1σ uncertainties, dash-dotted lines) corresponding to the optical mass function $f_{\text{opt}} = M_X \sin^3(i) / (1+q)^2 = 0.263 \pm 0.004 M_\odot$ and the mass ratio $q = M_C/M_X = A_X \sin(i)/A_C \sin(i) = 1.17 \pm 0.01$. The dashed lines give the inclination angle derived from the duration of the x-ray eclipse for different values of the radius R_C of HD 49798 (in units of $R_\odot$). The distances (in kpc) corresponding to the different values of R_C are also indicated.



49798 probably resulted from the evolution of an ~ 8 - to $9 M_\odot$ progenitor that began to fill its Roche lobe before helium ignition and is currently in a core helium-burning phase (2, 15). Alternatively, it could have a degenerate carbon-oxygen core and be in the shell helium-burning phase (6). The mass we derived for HD 49798 is consistent with both possibilities, and it rules out the case of a shell hydrogen-burning star resulting from a progenitor of mass less than $3.5 M_\odot$ that lost its hydrogen envelope while on the thermally pulsing asymptotic giant branch (6). The future evolution of HD 49798 will most likely lead to an increased mass transfer rate onto the white dwarf through Roche-lobe overflow during a semi-detached phase (15, 16). Theoretical evolutionary models of similar systems show that the transfer of helium-rich material can proceed at a rate of 10^{-6} to $10^{-5} M_\odot \text{ year}^{-1}$, leading to an accreted mass of a few tenths of solar masses (16). This would push the already massive white dwarf above the Chandrasekhar limit and possibly trigger a type Ia supernova explosion.

Our analysis confirms that the x-ray spectrum of RX J0648.0–4418 is very soft (4), being well fitted by a blackbody with temperature $kT = 39 \pm 1$ eV and an additional power law with photon index $\Gamma \sim 2$ that accounts for only a small fraction of the flux [see the supporting online material (SOM) text]. The x-ray luminosity in the 0.2- to 10-keV energy range is $\sim 2 \times 10^{31} (d/650 \text{ pc})^2 \text{ erg s}^{-1}$, where d is the source distance normalized to the value 650 ± 100 pc derived from optical observations (2) and consistent with the parallax $\mu = 1.16 \pm 0.63$ milli-arc sec measured with Hipparcos (17). The wind properties of HD 49798 were derived previously by modeling of the P Cygni profiles of the UV lines, which indicates a mass loss $\dot{M}$ in the range of 10^{-9} to $10^{-8} M_\odot \text{ year}^{-1}$ and a terminal wind velocity $V_{\text{wind}} = 1350 \text{ km s}^{-1}$ (5), attained at a distance of only ~ 1.7 stellar radii from the subdwarf. Orbiting HD 49798 at a distance $a = 4 \times 10^{11} (M_X + M_C)^{1/3} \text{ cm} \sim 8 R_\odot$, the white dwarf accretes a fraction $\eta \sim (R_A/2a)^2 \sim 0.0003$ of $\dot{M}$, where $R_A \sim 2 \times 10^{10} (1350 \text{ km s}^{-1}/V_{\text{wind}})^2$

cm is the accretion radius (18). The expected luminosity is thus $L_X = GM_X \eta \dot{M} / R_{\text{WD}} \sim 10^{31} (1350 \text{ km s}^{-1}/V_{\text{wind}})^4 (\dot{M}/10^{-9} M_\odot \text{ year}^{-1}) (3000 \text{ km}/R_{\text{WD}}) \text{ erg s}^{-1}$ (18), in good agreement with the observed value and implying that the x-ray luminosity of RX J0648.0–4418 can be interpreted in terms of accretion from the wind of the hot subdwarf companion. Mass accretion down to the white dwarf surface requires the magnetospheric radius, defined by balancing the magnetic pressure and the ram pressure of the infalling material, to be smaller than the co-rotation radius $R_{\text{cor}} = (GM_X P^2 / 4\pi^2)^{1/3} = 9 \times 10^8 \text{ cm}$. This translates into a magnetic dipole moment of $\sim 10^{29} (\dot{M}/10^{-8} M_\odot \text{ year}^{-1})^{15/16} (1350 \text{ km s}^{-1}/V_{\text{wind}})^{15/4} \text{ G cm}^3$ (19).

The high mass, fast spin, and possibly also low magnetic field (20) of RX J0648.0–4418 likely result from a previous evolutionary phase in which the accretion of mass and angular momentum took place at a much larger rate than what is currently observed. This system could thus be the white dwarf analog of low-mass x-ray binaries in which weakly magnetic neutron stars are spun up to short periods and shine as recycled millisecond radio pulsars when accretion onto them stops (21). A system probably undergoing such a transition from accretion-powered x-ray binary to millisecond radio pulsar has been reported very recently (22).

Most white dwarf masses are derived with indirect methods (23), such as surface gravity estimates obtained by spectral modeling, or the measurement of gravitational redshift (24). These methods, besides depending on models and assumptions, provide combined information on mass and radius; hence, the resulting masses cannot be used to independently determine the mass-radius relation. Because our mass determination is based on a direct dynamical measurement, RX J0648.0–4418 can be used to constrain the white dwarf's equation of state. The derived lower limit on the mass, together with the 13-s spin period, implies an upper limit of 6000 km on the white dwarf's radius, based

on simple rotational stability considerations (25). This limit is consistent with the classical predictions of zero-temperature stars (26), as well as recently derived mass-radius relations for massive oxygen-neon white dwarfs that predict a radius $R_{\text{WD}} \sim 3000 \text{ km}$ for $M_X = 1.2 M_\odot$ (27, 28).

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Supporting Online Material

www.sciencemag.org/cgi/content/full/325/5945/1222/DC1
SOM Text
Fig. S1
References

13 May 2009; accepted 23 July 2009
10.1126/science.1176252

Realization of an Excited, Strongly Correlated Quantum Gas Phase

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Ultracold atomic physics offers myriad possibilities to study strongly correlated many-body systems in lower dimensions. Typically, only ground-state phases are accessible. Using a tunable quantum gas of bosonic cesium atoms, we realized and controlled in one-dimensional geometry a highly excited quantum phase that is stabilized in the presence of attractive interactions by maintaining and strengthening quantum correlations across a confinement-induced resonance. We diagnosed the crossover from repulsive to attractive interactions in terms of the stiffness and energy of the system. Our results open up the experimental study of metastable, excited, many-body phases with strong correlations and their dynamical properties.

In many-body quantum physics, the interplay between strong interactions and confinement to a low-dimensional geometry amplifies the effects of quantum fluctuations and correlations. A remarkable example in one dimension is the Tonks-Girardeau (TG) gas, in which bosons with strong repulsive interactions minimize their interaction energy by avoiding spatial overlap and acquire fermionic properties (1, 2). Evidence for this ground-state phase was found using Bose-Einstein condensates (BECs) loaded into optical lattices (3, 4). Although many-body quantum systems are usually found in their ground-state phases, long-lived excited-state phases are responsible for some of the most striking physical effects; examples range from vortex lattices in superfluids to subtle topological excitations in spin liquids (5). However, the experimental realization of excited phases is difficult because these usually quickly decay from intrinsic effects or by coupling to the environment. In this context, cold atoms (3, 4, 6–12) may provide opportunities for the realization of long-lived, strongly interacting, excited many-body phases because of the excellent decoupling from the environment and the tunability of interactions via, for example, Feshbach resonances (FRs).

For an ultracold one-dimensional (1D) system of bosons, we prepared a highly excited many-body phase known as the super-Tonks-Girardeau (sTG) gas (13). In this highly correlated quantum phase, interactions are attractive, and rapid decay into a cluster-type ground state is possible, in principle. However, a surprising property of this many-body phase is its metastability. Attractive interactions strengthen correlations between particle positions and ensure, similar to an effective long-range repulsive interaction, that particles rarely come together. To realize this exotic phase, we observed and ex-

ploited a 1D confinement-induced resonance (CIR) (14, 15). This resonance allows us to first enter deeply into the repulsive TG regime in order to establish strong particle correlations and then to switch interactions from strongly repulsive to strongly attractive. The frequency ratio of the two lowest-energy collective modes (16) provides accurate diagnostics for the crossover from the TG to the sTG regime. In measuring particle loss and expansion, we studied the time evolution of the system through the crossover.

We tuned the strength of the interaction as characterized by the 3D scattering length a_{3D} by means of a magnetically induced FR (17). For a 1D system, a CIR arises and strongly modifies the 1D scattering properties when a_{3D} approaches the harmonic oscillator length $a_{\perp} = \sqrt{\hbar/(m\omega_{\perp})}$ of the transversal confinement with trap frequency

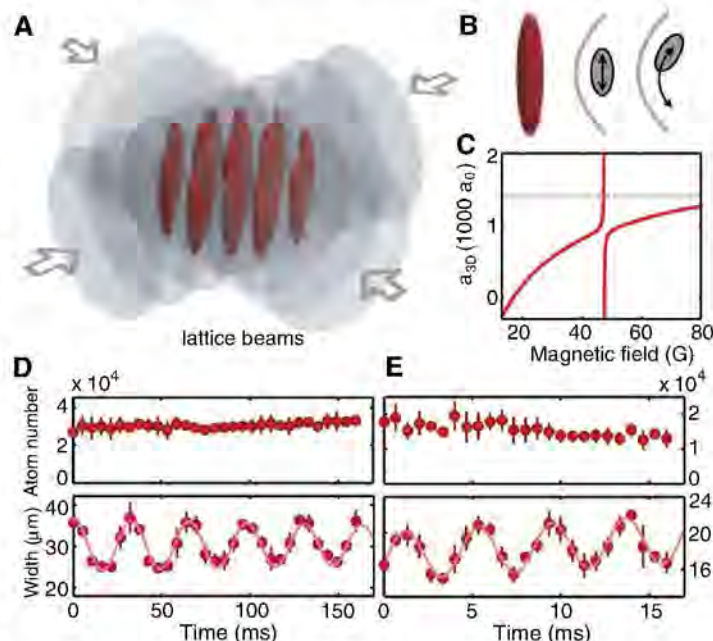
$\omega_{\perp}$ (14, 15). Here, m is the mass of the particles and $\hbar$ is Planck's constant divided by 2π . More precisely, the coupling constant g_{1D} of the 1D δ -function contact potential $U_{1D}(z) = g_{1D} \delta(z)$ behaves as (14)

$$g_{1D} = -\frac{2\hbar^2}{ma_{1D}} = \frac{2\hbar^2 a_{3D}}{ma_{\perp}^2} \frac{1}{1 - C a_{3D}/a_{\perp}} \quad (1)$$

where a_{1D} is the 1D scattering length defined by this equation and $C = 1.0326$ is a constant. Thus, the CIR allows tuning of g_{1D} . For values of a_{3D} less than but close to $a_{\perp}/C$ ($a_{3D} \lesssim a_{\perp}/C$), the coupling parameter g_{1D} is large and positive, and for $a_{3D} \gtrsim a_{\perp}/C$ it is large and negative, leading to an effectively attractive interaction. For homogenous systems with $g_{1D} > 0$, it is customary to characterize the strength of interactions by the Lieb-Liniger parameter $\gamma = g_{1D} m / (\hbar^2 n_{1D})$, where n_{1D} is the linear 1D density of the system (2, 6). The TG gas corresponds to the limit $\gamma \gg 1$ or $g_{1D} \rightarrow \infty$. As interactions are increased, the system becomes strongly correlated and is fully dominated by its kinetic energy. In previous experiments, without the capability to tune a_{3D} , a maximum of $\gamma \approx 5.5$ was achieved (4), whereas an effective strength $\gamma_{\text{eff}} \approx 200$ was reached with an additional shallow lattice potential along the longitudinal direction (3). In the former experiment, a saturation for the size and energy of the 1D system was observed, and in the latter experiment the momentum distribution was studied.

But what happens in the case of strong attractive interactions $g_{1D} \rightarrow -\infty$, that is, $a_{1D} \geq 0$? The ground state for a system of N attractively interacting bosons in 1D is a cluster state (18, 19),

Fig. 1. (A) Experimental setup. The lattice potential is created by two retro-reflected laser beams confining the atoms to an array of 1D tubes with equipotential surfaces shown in red. (B) Along each tube (left) we excited the lowest compressional mode (center) and compared its frequency to the dipole mode (right). (C) The strength of the interatomic interaction is adjusted by tuning the s-wave scattering length a_{3D} . The background scattering length rises gently from 0 to $1240 a_0$ when the magnetic field B is tuned from 17 to 76 G. Further tuning is possible near a FR at $47.78(1)$ G to absolute values beyond $4000 a_0$.



The dashed line indicates $a_{\perp}/C$ for a transversal trap frequency of $\omega_{\perp} = 2\pi \times 13.1$ kHz. (D and E) Typical data sets for the compressional mode in the TG and sTG regimes at $a_{3D} = 875(1) a_0$ and $a_{3D} = 2300(200) a_0$, respectively. The top panels show the atom number, and the bottom panels show the $1/e$ -cloud-width after time of flight. The solid lines in the bottom panels are sinusoidal fits (24), yielding the oscillation frequencies $\omega_c = 2\pi \times 30.6(3)$ Hz and $\omega_c = 2\pi \times 241(1)$ Hz, respectively.

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which one would expect in a cold-atom system to decay quickly via molecular channels. However, by crossing the CIR from the TG side (switching interactions from $g_{1D} = +\infty$ to $g_{1D} = -\infty$), an excited gaslike phase (the sTG gas) should be accessible (13). Is this excited phase stable; does it exist at all? The expectation is that the large kinetic energy inherited from the TG gas, in a Fermi pressure-like manner, prevents the gas from collapsing (20). This stability can most simply be inferred from a Bethe-ansatz solution to the Lieb-Liniger model with attractive interactions (20, 21). This ansatz yields for the energy per particle $E/N \approx \hbar^2 \pi^2 n_{1D}^2 / [6 m (1 - n_{1D} a_{1D})^2]$, corresponding to the energy of a gas of hard rods (1), for which a_{1D} represents the excluded volume. This results in a positive inverse compressibility and also in an increased stiffness of the system as long as $n_{1D} a_{1D}$ is sufficiently small. In this phase, the density cor-

relations are even stronger than in the TG gas because they show a power-law decay that is slower than for a TG gas (13), indicating an effective long-range interaction.

We realized the crossover all the way from a noninteracting gas via the 1D mean-field Thomas-Fermi (TF) regime to a TG gas and then to a sTG gas. We exploited the fact that our 1D systems possess weak harmonic confinement along the axial direction characterized by the confinement length $a_{\parallel}$. Whereas the frequency ω_D of the lowest dipole mode depends only on the confinement, the frequency ω_C of the lowest axial compressional mode is sensitive to the various regimes of interaction (16). For the noninteracting system, one expects $R = \omega_C^2/\omega_D^2 = 4$. This value then changes to $R = 3$ for weakly repulsive interactions in a 1D TF regime (7). For increasing positive interaction strength, R is expected to change smoothly to 4 when

entering the TG regime as the system becomes fermionized, hence effectively noninteracting. A rise beyond the value of 4, after crossing the CIR, would then constitute clear evidence for the sTG regime (13). As a_{1D} is further increased, the system will finally become unstable and R is expected to turn over and drop toward 0. For a harmonically confined system, the point of instability is reached when the overall length of the system of hard rods, $N a_{1D}$, becomes of the order of the size $\sqrt{N a_{\parallel}}$ for the wave function of N noninteracting fermions: $A \equiv N a_{1D} / (\sqrt{N a_{\parallel}}) \approx 1$. We use A^2 as an alternative parameter to γ so as to characterize the strength of the interaction because it accounts for the harmonic confinement.

We started from a 3D BEC with up to 2×10^5 cesium (Cs) atoms with no detectable thermal fraction in a crossed-beam dipole trap with magnetic levitation (22). Depending on the interaction regime to be studied, we then set the number of atoms in the BEC to values in the range of 1×10^4 to 4×10^4 by means of forced radio-frequency evaporation. To confine the atoms in 1D (that is, to freeze out transversal motion), we used a 2D optical lattice (12), which forms an array of vertically oriented elongated tubes with an aspect ratio that we set to values between 100 and 1000 (Fig. 1A). We occupied between 3000 and 6000 independent tubes with 8 to 25 atoms in the center tube. The interaction strength g_{1D} was controlled by magnetic tuning of a_{3D} by means of a combination of a broad and a narrow FR (Fig. 1C) with poles at $B = -11.1(6)$ G and $B = 47.78(1)$ G and widths of about 29.2 G and 164 mG, respectively (23). The broad resonance provides a slow variation of a_{3D} , allowing us to gently tune a_{3D} from 0 a_0 near 17.119 G to about 1240 a_0 near 76 G, whereas the narrow resonance allows us to tune a_{3D} to absolute values beyond 4000 a_0

Fig. 2. Transition from the noninteracting regime via the mean-field TF regime into the TG regime. The squared frequency ratio $R = \omega_C^2/\omega_D^2$ of the lowest compressional mode with frequency ω_C and the dipole mode with frequency ω_D serves as an indicator for the different regimes of interaction. For increasing interactions from $\gamma = 0$ to $\gamma \approx 500$, the system passes from the ideal gas regime ($R = 4$) to the 1D TF regime ($R \approx 3$) and then deeply into the TG regime ($R = 4$). The inset shows the transition from the noninteracting regime to the mean-field regime in more detail. The vertical error bars refer to 5E and the horizontal error bars reflect the uncertainty in determining a_{1D} and n_{1D} (24). The horizontal error bar on the data point at $\gamma = 0$ (not shown in the inset) is ± 0.03 .

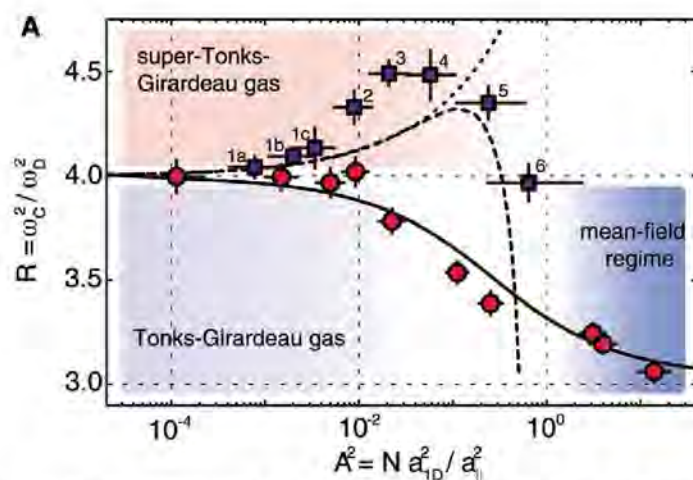
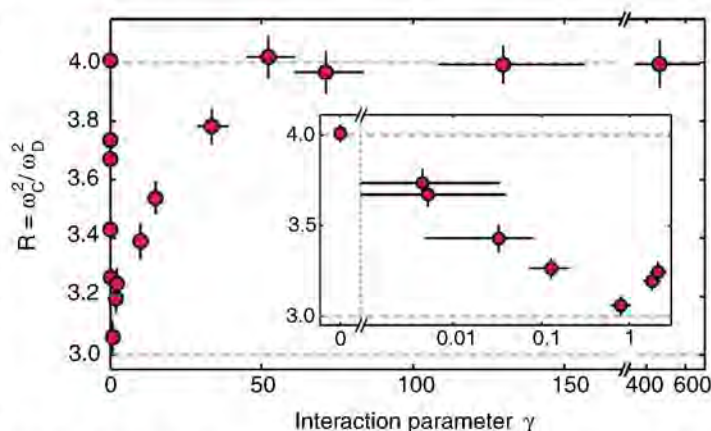
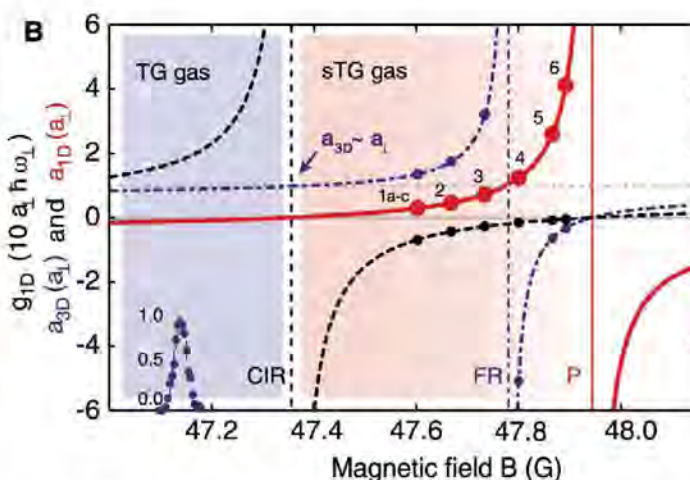


Fig. 3. (A) The ratio $R = \omega_C^2/\omega_D^2$ is plotted as a function of the interaction parameter $A^2 = N a_{1D}^2/a_{\parallel}^2$. The squares show the measurements in the attractive regime ($g_{1D} < 0$), providing evidence for the sTG gas. The circles show the transition from the TF to the TG regime ($g_{1D} > 0$); same data as in Fig. 2 for $\gamma > 1$. The solid line presents the theoretical data for $g_{1D} > 0$, and the dashed line presents the theoretical data for $g_{1D} < 0$, by Astrakharchik *et al.* (13). The dotted line corresponds to the model of hard rods. For reference, the measurements for $g_{1D} < 0$ are numbered. Data points 1c to 6 are taken at



$\omega_D = 2\pi \times 115.6(3)$ Hz. For data points 1a and 1b, the trap frequency is $\omega_D = 2\pi \times 22.4(1)$ Hz and $\omega_D = 2\pi \times 52.3(1)$ Hz, respectively. For all measurements in the sTG regime, $a_{\parallel} = 1346(5)$ a_0 . (B) The parameters a_{3D} (dashed-dotted), a_{1D} (solid), and g_{1D} (dashed) are plotted in the vicinity of the FR at 47.78(1) G. The horizontal dotted line indicates the value of $a_{\parallel}/C$. The pole of the CIR is at 47.36(2) G. a_{1D} has a pole (P) at 47.96(2) G. The bell-shaped curve at the bottom left indicates the atomic distribution as a function of the magnetic field determined from high-resolution microwave spectroscopy.

given our magnetic field control. We converted the applied magnetic field B into a_{3D} using the fit formula of (23). A magnetic field gradient, which we used to levitate the atomic sample (24), introduced a small spread in the value of a_{3D} across the sample.

To determine the oscillation frequencies ω_C and ω_D of the fundamental modes (Fig. 1B), we excited each mode separately at a given value of the magnetic field B (24) and let the atoms evolve for a varying amount of hold time. The distribution was then imaged in momentum space by taking an absorption picture after release and expansion. To avoid possible broadening effects due to interaction during the initial expansion, a_{3D} was set to 0 near $B = 17.119$ G at the moment of release. To extract the frequency, we determined for each hold time the axial $1/e$ -width of the distribution and then fit a damped sinusoid with linear offset to this data. Typical measurements of ω_C are shown in Fig. 1, D and E. Whereas the atom number remained constant for $g_{1D} > 0$, we observed some atom loss and a slight broadening of the distribution for attractive 1D interactions. In all parameter regimes, the 1D system was sufficiently stable to allow a reliable measurement of ω_C .

We show that we can tune the system from the noninteracting regime deeply into the repulsive TG regime (Fig. 2). In agreement with expectations, the value for $R = \omega_C^2/\omega_D^2$ first drops from 4 to 3 and then increases back to 4 as γ is tuned by means of the gently varying background scattering length. We found that the TG regime is fully reached for $\gamma > 50$. A further increase to values up to $\gamma \approx 500$ does not lead to changes for R . As a_{3D} approaches $a_{\perp}$, the divergence of g_{1D} according to Eq. 1 has to be taken into account when

determining γ (24). Heating of the system can be excluded because we can return to a 3D BEC without significant thermal background when ramping down the lattice potential.

The attractive regime was entered by crossing the CIR on the low-field wing of the 47.78 G FR. Here, a_{1D} is small and positive. The central results of this work are summarized in Fig. 3A and compared with the theoretical work of (13). We plot $R = \omega_C^2/\omega_D^2$ as a function of the interaction parameter A^2 . For reference, Fig. 3B plots a_{3D} , a_{1D} , and g_{1D} in the vicinity of the FR as a function of the magnetic field B . As the CIR is crossed and A^2 is increased, R rises beyond the value of 4. This provides clear evidence for the sTG regime because $R = 4$ is the maximal value for bosons with repulsive contact interaction. This increase is expected from the model of a gas of hard rods, and our data initially follow the prediction from this model. However, as A^2 is increased R reaches a maximum and then starts to drop. The maximum of about 4.5 is reached for $A^2 \approx 3 \times 10^{-2}$. The existence of the maximum is in qualitative agreement with the results obtained from Monte Carlo simulations (13). The theoretical prediction, however, underestimates the measured R . This is probably due to the local density approximation, which may not be applicable to our system with low particle numbers. For comparison, the results from Fig. 2 for $\gamma \geq 1$ are shown. $\gamma \approx 500$ corresponds to small values of $A^2 \approx 10^{-4}$. For this data, at higher particle numbers, there is excellent agreement with the theoretical prediction (Fig. 3, solid line) in the entire crossover from the mean-field regime to the TG regime (16).

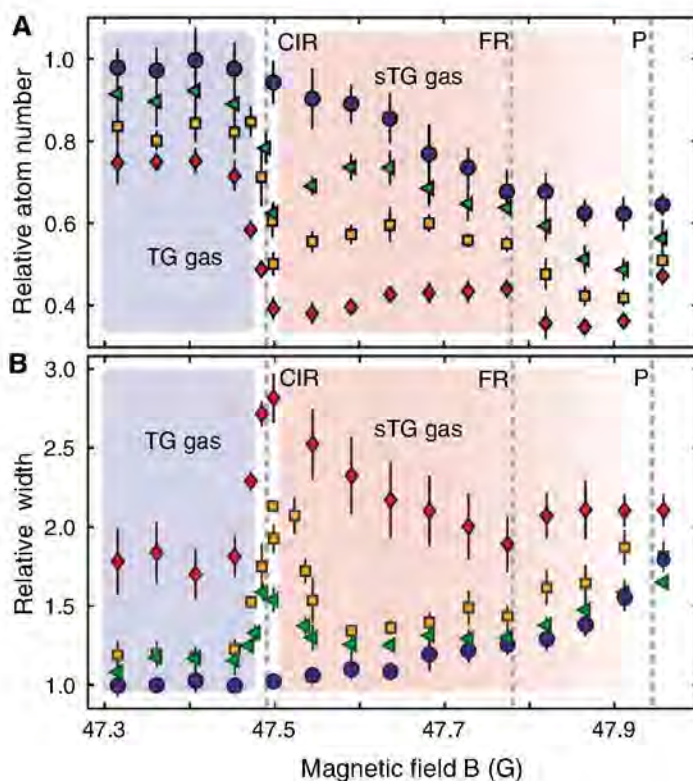
We studied the stability of the system in the crossover from the TG to the sTG regime and

found further evidence for the existence of the CIR by recording particle loss and measuring the axial width of the atomic cloud after release from the tubes. The axial width is a measure for the kinetic energy of the system because interactions are instantly switched off upon release. The conditions used were similar to those used for the measurements on the sTG regime presented in Fig. 3. The TG regime was entered adiabatically so as to avoid the excitation of collective modes. The system was prepared at $a_{3D} = 887(1) a_0$ at a magnetic field of $B = 42.77(2)$ G with about 11 atoms in the central tube. The magnetic field was then ramped to a specific value within 0.2 ms, and the sample was held at this value for a variable hold time τ from 10 to 200 ms. $a_{\perp}$ was set to $1523(6) a_0$. The results (Fig. 4) for different hold times τ in the tubes show that for $\tau = 10$ ms, corresponding to the timescale of the measurements in the sTG regime shown in Fig. 3, the transition from the TG to the sTG regime appears very smooth. There is essentially no particle loss when the system is deep in the TG regime and close to the CIR. The loss gradually increases in the attractive regime as one moves to larger values of B and toward the pole for a_{1D} . Correspondingly, the width of the sample exhibits a smooth behavior across the CIR, showing a slight increase for larger B . This behavior is consistent with the expectation of an increased energy in the sTG regime (13).

For longer hold times, the data for the atom number and the sample width develop distinct features at the calculated position of the CIR. Evidently, the system is in a transient state. For $\tau = 50$ ms, the number of remaining atoms shows a dip that correlates with a peak in the kinetic energy of the sample. Both features become more prominent and asymmetric for longer hold times ($\tau = 100$ and 200 ms). In comparison, no pronounced effects are visible at the pole of the FR for a_{3D} . Our results must be connected to the fact that the energy spectrum of the system changes dramatically across the CIR, from the TG to the sTG regime (19). The system acquires a deeply lying ground state together with a family of lower lying many-body excited states, potentially opening up new decay channels. Also, the CIR strongly modifies the two-body scattering problem, making formation of confinement-induced molecules in transversally excited trap states (14) possible.

The nontrivial time evolution observed in this system raises intriguing questions on possible coupling and decay mechanisms for strongly interacting, excited, many-body systems, in particular in the context of integrability of 1D systems (25). Our results offer an example of the counterintuitive effects that occur in many-body systems and open up the possibility to study the dynamical properties of strongly correlated systems with effective long-range interactions (26, 27) under conditions in which all parameters are tunable and, in fact, can be changed dynamically. Similar to magnetic FRs in atomic scattering, we expect the confinement-induced resonance demonstrated here to serve as

Fig. 4. Stability and kinetic energy in the TG and sTG regimes. (A) Relative number of atoms remaining and (B) relative $1/e$ -width along the axial direction after 10 ms expansion, after a hold time $\tau = 10, 50, 100$, and 200 ms (circles, triangles, squares, and diamonds, respectively) at a given magnetic field B . The position of the CIR, the pole of the FR, and the pole for a_{1D} (P) are as indicated. For these measurements, $a_{\perp} = 1523(6) a_0$ and $\omega_D = 2\pi \times 115.6(3)$ Hz. The atom number is normalized to the initial value of $1.7(1) \times 10^4$, and the width is normalized to the initial value in the TG regime.



a general tool to tailor interactions in 1D and possibly also in 2D systems (28), allowing for the further investigation of strongly correlated phases in the context of cold atomic gases.

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29. We thank S. Giorgini and C. Menotti for helpful discussions and for providing the theory curves shown in Fig. 3A. We are indebted to R. Grimm for generous support and to H. Häffner and his group for the loan of a charge-coupled device camera. We gratefully acknowledge funding by the Austrian Ministry of Science and Research (Bundesministerium für Wissenschaft und Forschung) and the Austrian Science Fund (Fonds zur Förderung der wissenschaftlichen Forschung) in the form of a START prize grant and by the European Union through the Specific Targeted Research Project FP7-ICT-2007-C project NAME-QUAM (Nanodesigning of Atomic and Molecular Quantum Matter) and within the framework of the EuroQUASAR collective research project Quantum-Degenerate Gases for Precision Measurements. R.H. is supported by a Marie Curie International Incoming Fellowship within the 7th European Community Framework Programme.

Supporting Online Material

www.sciencemag.org/cgi/content/full/325/5945/1224/DC1
Materials and Methods
References

4 May 2009; accepted 14 July 2009
10.1126/science.1175850

Complete Methods Set for Scalable Ion Trap Quantum Information Processing

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Large-scale quantum information processors must be able to transport and maintain quantum information and repeatedly perform logical operations. Here, we show a combination of all of the fundamental elements required to perform scalable quantum computing through the use of qubits stored in the internal states of trapped atomic ions. We quantified the repeatability of a multiple-qubit operation and observed no loss of performance despite qubit transport over macroscopic distances. Key to these results is the use of different pairs of $^9\text{Be}^+$ hyperfine states for robust qubit storage, readout, and gates, and simultaneous trapping of $^{24}\text{Mg}^+$ "re-cooling" ions along with the qubit ions.

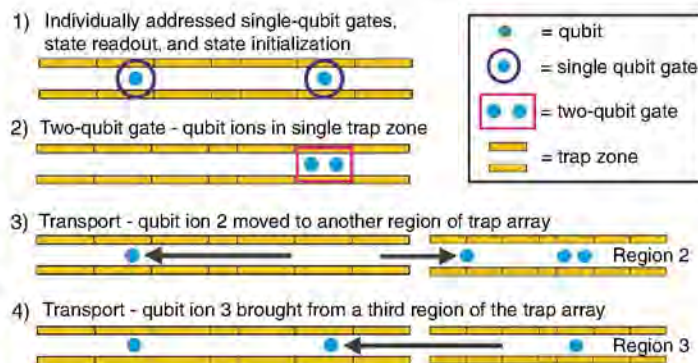
The long-term goal for experimental quantum information processing is to realize a device that involves large numbers of qubits and even larger numbers of logical operations (1, 2). These resource requirements are defined both by the algorithms themselves and the need for quantum error correction, which makes use of many physical systems to store each qubit (1, 3). The required components for building such a device are robust qubit storage, single- and two-qubit logic gates, state initialization, readout, and the ability to transfer quantum information between spatially separated locations in the processor (2, 4, 5). All of these components must be able to be performed repeatedly in order to realize a large-scale device.

One experimental implementation of quantum information processing uses qubits stored in the internal states of trapped atomic ions. A universal set of quantum logic gates has been demonstrated using laser addressing (6–8), leading to a number of small-scale demonstrations of quan-

tum information protocols, including teleportation, dense-coding, and a single round of quantum error correction (6). A major challenge for this implementation is now to integrate scalable techniques required for large-scale processing.

A possible architecture for a large-scale trapped-ion device involves moving quantum information around the processor by moving the ions themselves, in which the transport is controlled by time-varying potentials applied to electrodes in a multiple-zone trap array (5, 9, 10). The processor would consist of a large number of processing regions working in parallel, with other regions dedicated to qubit storage (memory). A general prescription for the required operations in a single processing region is the following (Fig. 1), which includes all of the elements necessary for universal quantum computation (11): (i) Two qubit ions are held in separate zones, allowing individual addressing for single-qubit gates, state readout, or state initialization. (ii) The ions are then combined in a single zone, and a two-qubit gate is performed. (iii) The ions are separated, and one is moved to another region of the trap array. (iv) A third ion is brought into this processing region from another part of the device. In this work, we

Fig. 1. Schematic of the sequence of operations implemented in a single processing region for building up a computation in the architecture of (5, 9). A large-scale device would involve many of these processing regions performing operations in parallel along with additional regions for memory. Generalized operations would use this block structure repeatedly, with perhaps some of the steps omitted.



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implemented in a repeated fashion all of the steps that must be performed in a single processing region in order to realize this architecture.

Some elements of this architecture have been demonstrated in previous experiments (6, 12), which involved transport of ions in a multiple-zone trap. However, these experiments did not involve the use of techniques required for building a large-scale device, limiting the size of algorithms that could be performed. Primary limiting factors for these experiments were the loss of qubit coherence, caused by interaction with the fluctuating magnetic field environment, and motional excitation, which degrades the fidelity of subsequent two-qubit gates because of the finite

wavelength of the gate control fields (13). Motional excitation occurs as a result of imperfect control during transport and noisy electric fields emanating from the electrode surfaces (6). In this work, we robustly stored qubits using a pair of energy eigenstates in the $^9\text{Be}^+ 2s^2S_{1/2}$ hyperfine manifold (Fig. 2), whose energy separation does not depend on the magnetic field to first order. For the $^9\text{Be}^+$ “qubit” ions used here, this condition is met at a magnetic field of 0.011964 T for the “memory” qubit states $|1\rangle \equiv |F=1, M_F=0\rangle$ and $|0\rangle \equiv |F=2, M_F=1\rangle$ (the states are labeled by using the total angular momentum quantum numbers F and M_F). The insensitivity to magnetic-field changes is crucial for preserving coherence in the

presence of ambient temporal field fluctuations (14) and also greatly suppresses phase shifts caused by spatial variations in the average field experienced by an ion as it is transported throughout the multiple-zone trap array. We removed motional excitation before each two-qubit gate by recoiling “refrigerant” $^{24}\text{Mg}^+$ ions that were trapped along with the qubit ions. Laser-cooling this second species sympathetically cools the first through the strong Coulomb interaction between the ions (15–18).

A benchmark for scalability in this implementation is the repeated performance of a complete set of one- and two-qubit logic gates combined with quantum information transport. We demonstrated repeatability of a unitary transformation $\hat{U}$, which involves four single-qubit gates, a two-qubit gate, and transport over 960 μm (the sequence for $\hat{U}$ is shown in Fig. 3A). Ideally, $\hat{U}$ implements the operation

$$\hat{U} = -\frac{e^{-i\pi/4}}{\sqrt{2}} \begin{pmatrix} -1 & 0 & 0 & i \\ 0 & 1 & i & 0 \\ 0 & i & 1 & 0 \\ i & 0 & 0 & -1 \end{pmatrix} \quad (1)$$

Fig. 2. Hybrid qubit storage in the $^9\text{Be}^+ 2s^2S_{1/2}$ hyperfine levels. The states are labeled with the total angular momentum quantum numbers F and M_F . $|1\rangle, |0\rangle$ are the qubit states used for single-qubit gates and transport, and $|1_G\rangle, |0_G\rangle$ are used for two-qubit gates. For detection, the $|1, -1\rangle$ and $|2, 2\rangle$ states are used. At the applied magnetic field ($B \approx 0.011964$ T), the frequencies for transitions between pairs of states with the same F are well resolved.

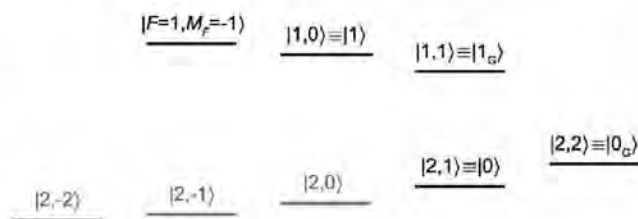
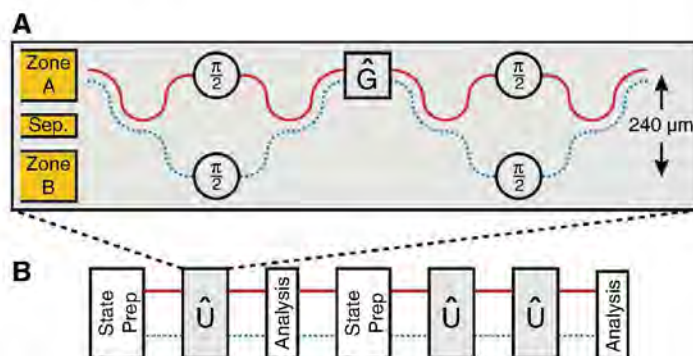
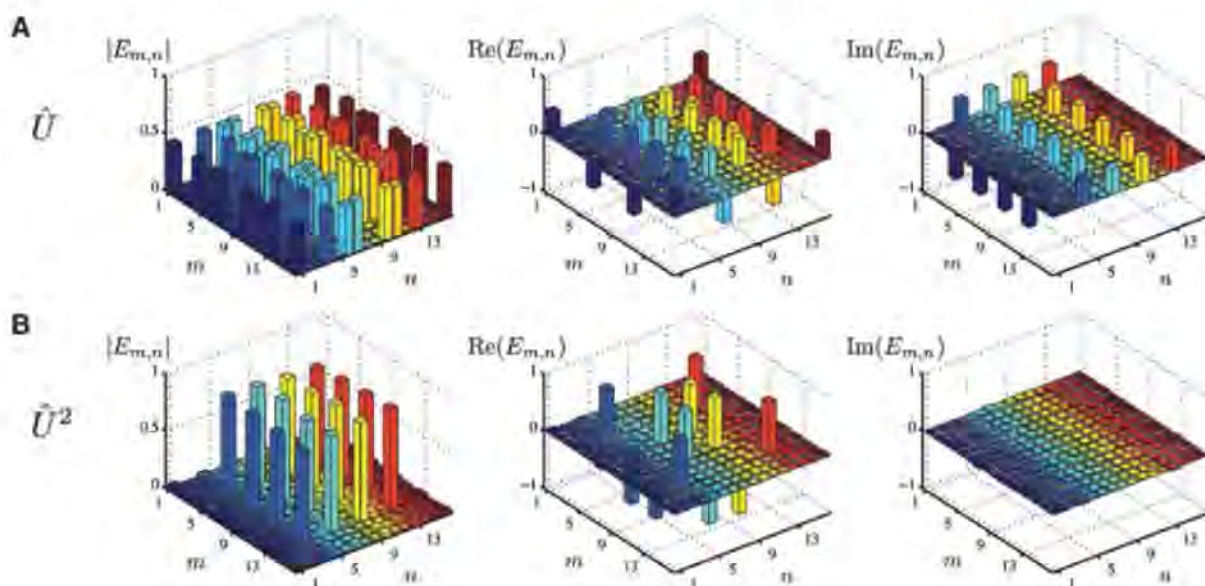


Fig. 3. (A) Schematic of the qubit ion trajectories (the solid red line indicates qubit 1 and the dotted blue line indicates qubit 2) and gate operations used to implement $\hat{U}$. The single-qubit rotations are $\pi/2 \equiv R(\pi/2, 0)$ (Eq. 2). The two-qubit gate implements $\hat{G} = D[1, i, i, 1]$. (B) Full sequence used to perform process tomography on $\hat{U}$ and $\hat{U}^2$. This sequence was repeated 350 times for each setting of preparation/analysis.



in the $|11\rangle, |10\rangle, |01\rangle, |00\rangle$ basis. We directly compared experimental implementation of $\hat{U}$ and $\hat{U}^2$ using quantum process tomography (19). Process tomography requires the process under investigation to be applied to 16 input states followed by measurement in nine orthogonal bases (20). The input states were prepared by use of a combination of optical pumping and single-qubit operations, with the latter performed on each qubit individually. The analysis also requires individual single-qubit rotations followed by individual state-measurement of the qubits. The experiment therefore realizes all of the basic components illustrated in Fig. 1. We directly compared $\hat{U}$ and $\hat{U}^2$ by running the experimental sequence for a given input/output combination on $\hat{U}$ and $\hat{U}^2$ sequentially (Fig. 3B), making the comparison of the two

Fig. 4. Reconstructed process matrix for (A) $\hat{U}$ and (B) two repetitions of $\hat{U}$. The map $\mathcal{E}(|i\rangle\langle j|)$ produces a matrix E_{kl} for each element $|i\rangle\langle j|$. Hence, elements of the matrix E are labeled by $m = 4(i-1) + k$, $n = 4(j-1) + l$, where the factor 4 results from the size of the two-qubit state space. For example, the $|11\rangle\langle 00|$ ($i=1, j=4$) element of an input density matrix is mapped to $\mathcal{E}(|11\rangle\langle 00|)$, a 4 by 4 block of E given by $m \in [1, 4]$ and $n \in [13, 16]$. The position of each peak is in agreement with the theoretical prediction.



robust against long-term drifts in experimental parameters. For each input/analysis combination, we repeated this sequence 350 times.

The experiment used two $^9\text{Be}^+$ and two $^{24}\text{Mg}^+$ ions trapped in a six-zone linear Paul trap (12). Each $^9\text{Be}^+$ ion was used to store one qubit and was accompanied at all times by a $^{24}\text{Mg}^+$ refrigerant ion, which was used for sympathetic cooling. The ion order was initialized to $^9\text{Be}^+ - ^{24}\text{Mg}^+ - ^{24}\text{Mg}^+ - ^9\text{Be}^+$ at the start of the experimental sequence and remained in this order throughout (21).

We performed coherent manipulations of the internal and motional states of the ions using laser-induced stimulated Raman transitions (9). Single-qubit gates were implemented in the basis $|1\rangle, |0\rangle$ by means of resonant Rabi flopping, applying the rotation

$$R(\theta, \phi) = \begin{pmatrix} \cos(\theta/2) & -ie^{-i\phi}\sin(\theta/2) \\ -ie^{i\phi}\sin(\theta/2) & \cos(\theta/2) \end{pmatrix} \quad (2)$$

where θ is proportional to the Raman pulse duration and ϕ was chosen by adjusting the relative phase of the Raman light fields at the ion. We individually addressed the two-qubit ions by holding them in two trap zones 240 μm apart and switching the laser beams between zones.

To implement two-qubit gates, we first combined all of the ions into a single zone. The four-ion linear chain exhibits four axial vibrational normal modes caused by the Coulomb coupling between ions (21). After recombination, these modes contain significant excess energy, which is mainly caused by imperfect control of the potentials used during separation and recombination. Therefore, before each two-qubit gate we cooled each mode to near the quantum ground state ($\langle n \rangle \sim 0.06$) using a combination of Doppler cooling and resolved sideband cooling on the $^{24}\text{Mg}^+$ ions (15, 22). The cooling light only interacts with $^{24}\text{Mg}^+$, leaving the qubits stored in $^9\text{Be}^+$ intact (15).

The composite two-qubit gate makes use of a geometric phase gate (7) to implement $\hat{G} = D[(1, i, i, 1)]$, where $D[\mathbf{v}]$ is a diagonal matrix with the vector $\mathbf{v}$ on the diagonal. The phase acquired by the $|10\rangle$ and $|01\rangle$ states was obtained by means of transient simultaneous excitation of the two highest-frequency normal modes by use of a state-dependent optical dipole force (22). The state dependence of this force is derived from a differential light shift between the two-qubit states, which is highly suppressed for field-independent transitions (14, 23). We thus used a hybrid scheme for qubit storage, mapping the qubits into a different state manifold for the two-qubit gate (22, 24, 25). Before applying the optical dipole force, we transferred each qubit into a pair of states with a sizeable differential light shift—the “gate” manifold $|1_G\rangle \equiv |1, 1\rangle, |0_G\rangle \equiv |2, 2\rangle$ (Fig. 2). After applying the state-dependent force, we reversed this transfer and the ions were again separated (22). The gate manifold is sensitive to

magnetic-field fluctuations, which can lead to qubit dephasing. We suppressed these effects using spin-echo techniques (21).

We used quantum process tomography to characterize our implementation of the unitary operation $\hat{U}$, including any experimental imperfections (19, 20). The evolution of the qubit system (including that caused by undesired interactions with the environment) is described by a completely positive linear map $\rho_{\text{out}} = \mathcal{E}_{\hat{U}}(\rho_{\text{in}})$ (19) on the input density matrix $\rho_{\text{in}} = \sum_{i,j} c_{ij} |i\rangle\langle j|$, where the c_{ij} are complex numbers and i, j are labels that each run over the eigenstates $|11\rangle, |10\rangle, |01\rangle$, and $|00\rangle$. Following the method described in (26), we represent the map with a 16 by 16 matrix

$$E_{\hat{U}} = \sum_{i,j} |i\rangle\langle j| \otimes \mathcal{E}_{\hat{U}}(|i\rangle\langle j|) \quad (3)$$

In order to extract this process matrix, we experimentally applied the process to 16 input states made up of tensor products of the states $|1\rangle, |0\rangle, (|0\rangle - |1\rangle)/\sqrt{2}$, and $(|0\rangle + i|1\rangle)/\sqrt{2}$. For each output-density matrix, we applied nine sets of rotations, which allowed us to measure the expectation values of the operators $\sigma_x \otimes \sigma_x$, where the $\sigma_{s,t}$ run over the Pauli matrices I, σ_x, σ_y , and σ_z . Our state readout performs a projective measurement in the Z basis on each ion independently. We first transferred population from $|0\rangle$ to $|2, 2\rangle$, and from $|1\rangle$ to $|1, -1\rangle$ and subsequently drove the cycling transition $2s^2S_{1/2} |2, 2\rangle \leftrightarrow 2p^2P_{3/2} |3, 3\rangle$ for 200 μs , in which $|2, 2\rangle$ strongly fluoresces and $|1, -1\rangle$ does not (22). We collected a small fraction of the emitted photons on a photomultiplier tube. We ran the sequence shown in Fig. 3B 350 times for each of the 16 input states and nine measurement rotations. The process matrix was obtained directly from the recorded photon counts and measurement/preparation settings by means of a maximum-likelihood method that ensures that the reconstructed process matrix is physical (26).

Experimentally obtained process matrices for one and two applications of $\hat{U}$ are shown in Fig. 4. From the reconstructions, we can calculate various measures of the fidelity with which the processes were implemented. A direct comparison between experimental results and the ideal case is given by the entanglement fidelity $F \equiv \text{Tr}(E_{\text{ideal}} E)/16$ (27). We found $F_{\hat{U}} = 0.922(4)$ for a single application of $\hat{U}$ and $F_{\hat{U}^2} = 0.853(5)$ for two applications [error estimates are the SE on the mean obtained from parametric bootstrap resampling (22)]. As an additional measure of operation fidelity, we took the mean $\bar{f}$ of the fidelity $f(\rho_{\text{ideal}}, \rho_E) \equiv [\text{Tr}(\sqrt{\rho_{\text{ideal}} \rho_E \rho_{\text{ideal}}})]^2$ (28) between the output-density matrices obtained from the ideal and experimental processes for an unbiased set of 36 input states (formed from the eigenstates of $\sigma_x \otimes \sigma_x$, where $\sigma_{s,t}$ run over σ_x, σ_y , and σ_z). We obtained a mean state fidelity of $\bar{f}_{\hat{U}} = 0.940(4)$ for $E_{\hat{U}}$ and $\bar{f}_{\hat{U}^2} = 0.890(4)$ for $E_{\hat{U}^2}$. We can compare these values with the entanglement fidelities by using the relation $\bar{f} = (4F + 1)/5$ (27) and see that they are consistent.

To compare the performance of a second application of $\hat{U}$ relative to the first, we can compare its experimental repetition $\mathcal{E}_{\hat{U}^2}(\rho_{\text{in}})$ to a numeric repetition of the experimental map $\mathcal{E}_{\hat{U}}(\rho_{\text{in}})$; that is, to $\mathcal{E}_{\hat{U}^2}(\rho_{\text{in}}) \equiv \mathcal{E}_{\hat{U}}[\mathcal{E}_{\hat{U}}(\rho_{\text{in}})]$. Evaluating the fidelities for each against the ideal case yields $F_{\hat{U}^2}/F_{\hat{U}} = 1.003(13)$ and $\bar{f}_{\hat{U}^2}/\bar{f}_{\hat{U}} = 1.004(10)$, indicating that the operation fidelity is the same for each application of $\hat{U}$. We can also make a direct comparison between the processes performed by our implementation of $\hat{U}$ and $\hat{U}^2$ by taking the mean fidelity between $\rho_{\hat{U}\hat{U}} = \mathcal{E}_{\hat{U}}[\mathcal{E}_{\hat{U}}(\rho_{\text{in}})]$ and $\rho_{\hat{U}^2} = \mathcal{E}_{\hat{U}^2}(\rho_{\text{in}})$ for the 36 input states. We find $\bar{f}(\rho_{\hat{U}\hat{U}}, \rho_{\hat{U}^2}) = 0.987(3)$. Although this number is not unity, as might be expected, the deviation can be ascribed to bias in the maximum-likelihood reconstruction method for a finite sample size (22). Our results are thus consistent with the same operation being performed by the experiment for each application of $\hat{U}$.

Sources of error in our system arise primarily from spontaneous photon scattering ($\sim 1.5\%$ per $\hat{U}$) (29) and intensity fluctuations of the Raman light fields at the percent level. In order to characterize the loss of fidelity caused by single-qubit rotations, we applied process tomography to the experimental sequence but without the state-dependent force pulses. In this case, the ions are always in a product state, and the process matrix for each can be obtained independently. The resulting process matrices have a mean state fidelity relative to the ideal case of 0.97 for a single run of the sequence (which uses eight rotations per ion, including qubit-manifold transfer and spin-echo pulses). During the two-qubit gate, the spin states are entangled with the motion. From separate measurements of motional coherence, we estimate the infidelity from this source to be less than 1×10^{-3} .

Many challenges remain before large-scale ion trap quantum information processing becomes a reality, including increasing fidelities to those required for fault-tolerant quantum error correction (1, 3) and meeting the considerable technical challenge of controlling ions in large multidimensional trap arrays (10). Both of these challenges could potentially contain problems that have not been considered here and may require combining our approach with alternative methods, for instance entanglement distribution by means of photonic networks (30). Nevertheless, the combination of techniques demonstrated here includes all of the basic building blocks required in this architecture and opens up new possibilities for quantum information processing as well as state and process engineering.

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31. This work was supported by Intelligence Advanced Research Projects Activity and the NIST Quantum Information Program. J.P.H. acknowledges support from a Lindemann Trust Fellowship. We thank E. Knill for helpful discussions, J. J. Bollinger for technical assistance, and Y. Colombe for comments on the manuscript. This paper is a contribution by NIST and not subject to U.S. copyright.

Supporting Online Material

www.sciencemag.org/cgi/content/full/1177077/DC1

Materials and Methods

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1 June 2009; accepted 8 July 2009

Published online 6 August 2009;

10.1126/science.1177077

Include this information when citing this paper.

A Sulfilimine Bond Identified in Collagen IV

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Collagen IV networks are ancient proteins of basement membranes that underlie epithelia in metazoa from sponge to human. The networks provide structural integrity to tissues and serve as ligands for integrin cell-surface receptors. They are assembled by oligomerization of triple-helical protomers and are covalently crosslinked, a key reinforcement that stabilizes networks. We used Fourier-transform ion cyclotron resonance mass spectrometry and nuclear magnetic resonance spectroscopy to show that a sulfilimine bond ($-S=N-$) crosslinks hydroxylysine-211 and methionine-93 of adjoining protomers, a bond not previously found in biomolecules. This bond, the nitrogen analog of a sulfoxide, appears to have arisen at the divergence of sponge and cnidaria, an adaptation of the extracellular matrix in response to mechanical stress in metazoan evolution.

Collagen IV networks are ancient proteins of basement membranes, a specialized form of extracellular matrix, that underlie epithelia in metazoa from sponge to human. The networks confer structural integrity to tissues; serve as scaffolds for the assembly of other macromolecular components; and serve as ligands for integrin cell-surface receptors that mediate cell adhesion, migration, growth, and differentiation (1–3). The networks participate in signaling

events in *Drosophila* development (4) and in the clustering of receptors in the development of mammalian neuromuscular junction (5), and they are involved in autoimmune and genetic diseases (6–8). The networks are assembled by oligomerization of triple-helical protomers by end-to-end associations and by intertwining of triple helices (9, 10). At the C terminus, two protomers associate through their trimeric noncollagenous (NC1) domains, forming a hexamer structure. The protomer-protomer interface is covalently crosslinked, a key reinforcement that strengthens the structural integrity of networks. In the case of humans, the crosslink also confers immune privilege to the collagen IV antigen of Goodpasture autoimmune disease (11, 12).

The chemical nature of these crosslinks has been the subject of numerous investigations for

2 decades; yet, the identity of the covalent bond has remained unknown. Initially, the crosslinks were identified as disulfide bonds (13), which were subsequently ruled out by the x-ray crystal structure of NC1 hexamers (14, 15). Electron density maps suggested connectivity between methionine-93 (Met⁹³) and lysine-211 (Lys²¹¹) at the interface of adjoining protomers (15); however, the connectivity is gradually degraded by x-rays, rendering precise characterization a challenge for structural analysis by crystallography (16, 17). By using mass spectrometry (MS) analyses of crosslinked tryptic (Tp) peptides and smaller crosslinked post-proline endopeptidase (PPE) peptides, both derived from the $\alpha1\alpha2\alpha1$ collagen IV network of placenta, we found that Lys²¹¹ is modified to hydroxylysine (Hyl²¹¹) and that Hyl²¹¹ is covalently linked to Met⁹³, forming a *S*-hydroxylysyl-methionine (sHM) crosslink (18). In the $\alpha3\alpha4\alpha5$ network (11), we found that the sHM crosslink connects the $\alpha3$ and $\alpha5$ NC1 domains but that the $\alpha4$ NC1 domains are connected by a *S*-lysyl-methionine crosslink involving Lys²¹¹ instead of Hyl²¹¹, indicating that this posttranslational modification is not a requirement for crosslink formation (11). The nature of the bond linking Met⁹³ and Hyl²¹¹ could not be determined at that time because the observed difference of one mass unit between the uncrosslinked and crosslinked peptides fell within experimental error.

Herein, we deduce the chemical nature of the bond by using Fourier transform ion cyclotron resonance (FTICR) MS (19), which can achieve very high mass accuracy [e.g., < 2 parts per million (ppm), about ± 0.001 mass units for a peptide with a mass of ~ 5000], and nuclear magnetic resonance (NMR) spectroscopy to

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Table 1. Mass values for the crosslinked Tp peptides from the $\alpha1NC1$ - $\alpha1NC1$ dimer. The average of the observed peptide mass is indicated with an asterisk.

Z	Theoretical mass	Observed ion (m/z)	Observed peptide mass	Difference (theo. – obs.)
+4		1253.6204	5010.473 $\pm$ 0.008	
+5		1003.1014	5010.483 $\pm$ 0.022	
+6		836.0806	5010.459 $\pm$ 0.009	
	5012.488		5010.471 $\pm$ 0.022*	2.017

analyze crosslinked Tp peptides derived from the α INC1- α INC1 dimer. Table 1 shows the mass values for the multiple-charge states (+4, +5, and +6) of the crosslinked Tp peptide. For each charge state, 10 consecutive scans were averaged to obtain the observed monoisotopic mass value of 5010.471 ± 0.022 (SEM) (Table 1). This value is 2.017 mass units lower than the theoretical total mass (5012.486) of the two constituent tryptic peptides, a Met⁹³-containing peptide (T-3599.688) and a Hyl²¹¹-containing peptide (T-1412.777), which were previously described (18).

To determine the location of modifications that lead to the loss of 2.017 mass units, we analyzed the tryptic complex by collision-induced dissociation (CID) MS³ (MS/MS/MS) fragmentation analysis. The mass-to-charge ratio (m/z) of

1003.1014 (+5) ion was selected for MS² fragmentation, which generated a $m/z = 730.399$ ion, corresponding to the T-1412.799 peptide plus 45.984 mass units, and a $m/z = 1184.900$ ion, corresponding to the T-3599.689 peptide that lost 48.013 mass units (Fig. 1A). To locate these mass changes to specific residues, we selected the $m/z = 730.3994$ and $m/z = 1184.900$ ions for further CID (MS³). The y and b series of the spectra confirm not only the sequence of the T-1412.799 peptide but also that the location of the mass change of +45.984 corresponds to the side chain of Hyl²¹¹ (fig. S1A). The MS³ fragmentation profile for the $m/z = 1184.900$ ion also verified the peptide sequence and localized the loss of 48.013 mass units to Met⁹¹ or Met⁹³ (fig. S1B). A smaller crosslinked PPE peptide

complex derived from the crosslinked Tp peptides (fig. S2) confirmed that the loss of 48.013 mass units is localized to the side chain of Met⁹³. An analogous fragmentation has been observed in methionine sulfoxide-containing peptides that undergo concomitant neutral loss of methane sulfenic acid (CH₃SOH) (20, 21). In this case, however, this group remains attached to the side chain of Hyl²¹¹, demonstrating the covalent nature of the interaction between Met⁹³ and Hyl²¹¹. These findings for the crosslink in the α INC1- α INC1 dimer are identical to that for the α 2NC1- α 2NC1 dimer (fig. S3).

An overlay of correlation spectroscopy (COSY) and heteronuclear multiple quantum correlation (¹H-¹³C HMQC) spectra of the crosslinked Tp peptides was used to evaluate the chemical shift of the methyl group of Met⁹³ (Fig. 1B and fig. S4A). The sequences of crosslinked Tp peptides have a total of 25 methyl groups, three of which belong to Met⁸⁹, Met⁹¹, and Met⁹³. Typical proton chemical shift for methyl groups in peptides of Leu, Thr, Iso, and Ala is between 0 and 1.5 ppm and that of Met is about 2.1 ppm. Eighteen out of 22 total methyl groups of Leu, Thr, Ile, and Ala were identified within the expected chemical shift range in the HMQC spectrum (fig. S4B). An edited heteronuclear single quantum correlation (HSQC) analysis was carried out to identify the methyl groups of Met⁸⁹, Met⁹¹, and Met⁹³. In the chemical shift range expected for Met, two signals were observed: one at ¹H 1.9 ppm/¹³C 14 ppm and the other at ¹H 1.7/¹³C 24 ppm, presumably corresponding to Met⁸⁹ and/or Met⁹¹ (Fig. 1C). In addition, two downfield-shifted methyl resonances were observed that had atypical chemical shifts at ¹H 2.9 ppm/¹³C 30 ppm and ¹H 2.6 ppm/¹³C 36 ppm. These features likely correspond to the methyl group of Met⁹³ in two different chemical environments, such as cyclic or alternate chiral forms of the crosslink (fig. S5).

The collective evidence from the MS and NMR indicates the existence of a sulfilimine bond in the crosslinked Tp peptides (Fig. 2A). MS analysis revealed that the linkage between Met⁹³ and Hyl²¹¹ is characterized by the loss of two hydrogen atoms, which is consistent with oxidation resulting in a double bond that connects the sulfur atom of Met⁹³ and the nitrogen atom of Hyl²¹¹ (Fig. 2A). The MS² fragmentation of the tryptic complex can be explained by a concerted process in which a β -hydrogen is taken up by the nitrogen, which leads to the cleavage of the bond between sulfur and γ -carbon of Met⁹³ (Cope elimination) (22). This syn elimination is also observed in the fragmentation of methionine sulfoxide and is analogous to the Cope elimination of amine oxides. The resulting products, an olefin fragment and a methylsulfenamide fragment, are shown in Fig. 2A. The sulfilimine structure is further supported by a down-shifted methyl peak in the NMR spectrum of the crosslinked Tp peptides, which is consistent with the shift of the methyl group of S-methylisothiazolidinium nitrate (¹H 2.78 ppm).

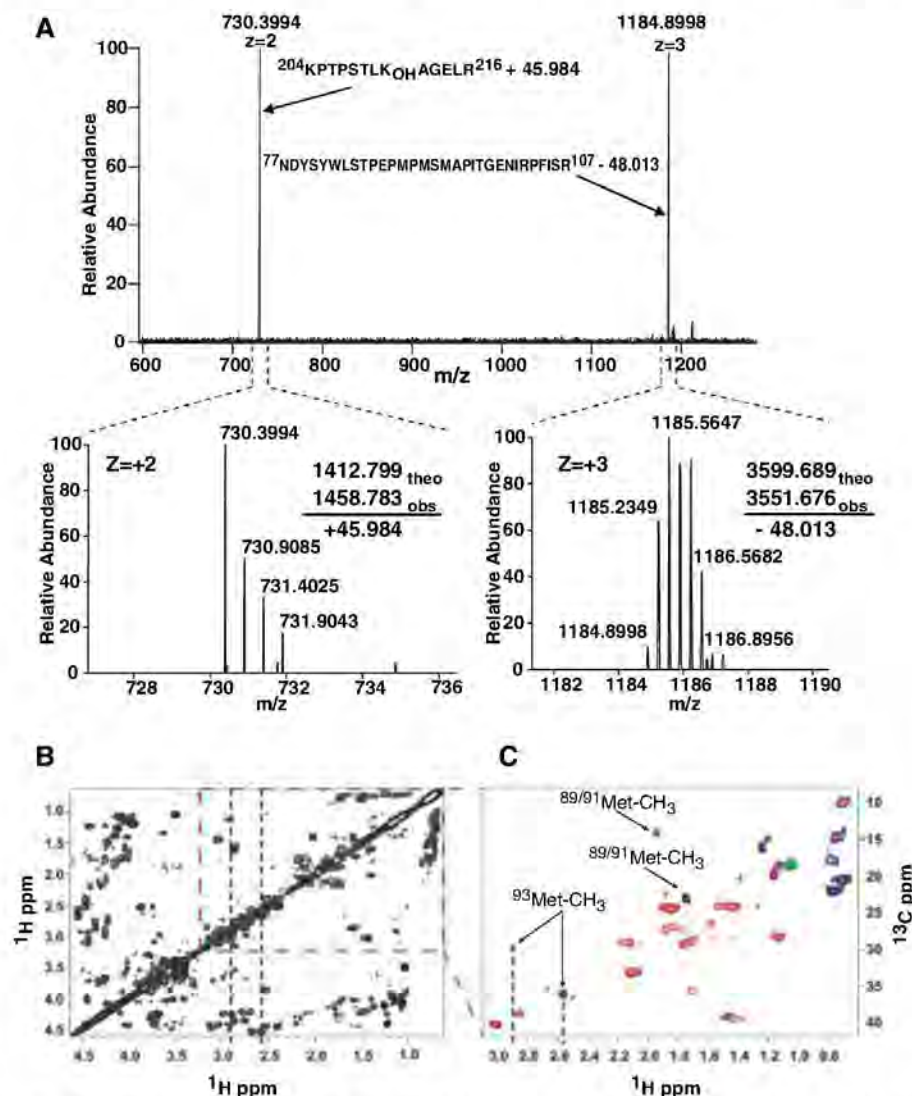


Fig. 1. Mass spectrometric and NMR analyses of the crosslinked Tp peptides from the α INC1- α INC1 dimer. (A) MS² spectrum showing the fragmentation by CID of the $m/z = 1003.1014$ (+5) ion (Table 1). The bottom graphs show the isotopic envelope for each ion and the mass difference of each fragment with respect to the uncrosslinked peptides. (B) NMR studies showing the ¹H-¹H COSY spectrum in 50 mM phosphate buffer, pH = 7.0, 20°C. (C) Edited ¹H-¹³C HSQC spectrum of an expanded portion of (B) in which the methyl groups of Val, Leu, Thr, Iso, and Met are expected. Correlation peaks are color-coded according to multiplicity edited HSQC spectra, optimized for correlation selection of CH₂ (red), CH/CH₃ (blue), and CH (green) only. Several peaks contain overlapping signals from CH₃ and CH groups and are indicated in purple.

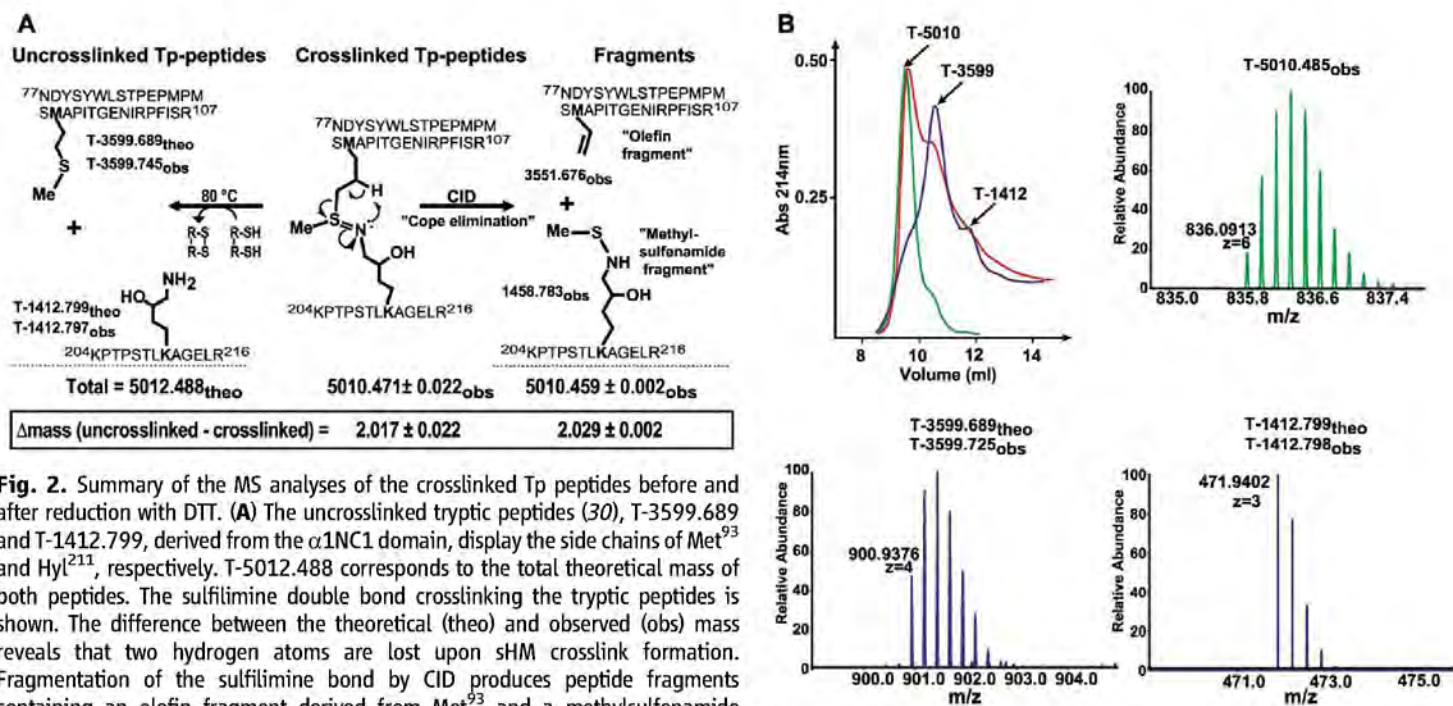
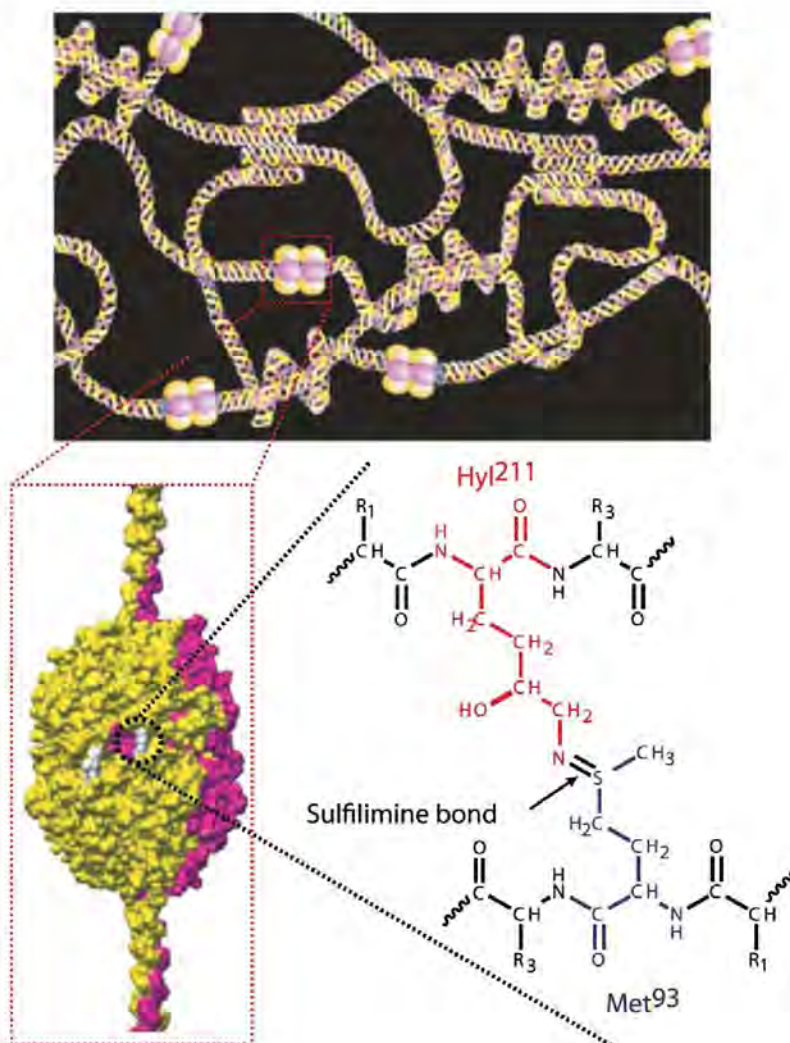


Fig. 2. Summary of the MS analyses of the crosslinked Tp peptides before and after reduction with DTT. **(A)** The uncrosslinked tryptic peptides (30), T-3599.689 and T-1412.799, derived from the $\alpha 1\text{NC1}$ domain, display the side chains of Met⁹³ and Hyl²¹¹, respectively. T-5012.488 corresponds to the total theoretical mass of both peptides. The sulfilimine double bond crosslinking the tryptic peptides is shown. The difference between the theoretical (theo) and observed (obs) mass reveals that two hydrogen atoms are lost upon sHM crosslink formation. Fragmentation of the sulfilimine bond by CID produces peptide fragments containing an olefin fragment derived from Met⁹³ and a methylsulfenamide fragment derived from Hyl²¹¹ as a result of the Cope elimination event in the gas phase. However, chemical reduction with DTT, formally involving addition of two H atoms, severs the sulfilimine link and recovers Met⁹³ and Hyl²¹¹, as indicated below. **(B)** Crosslinked Tp peptides were separated by gel filtration chromatography before (green) and after incubation in 100 mM DTT at room temperature (red) and 80°C (blue). The arrows indicate the identity of each chromatographic peak as revealed by MS analysis.

Fig. 3. Proposed chemical structure of the sHM crosslink. **(Top)** Schematic of the $\alpha 1\alpha 2\alpha 1$ collagen IV network illustrating the interaction between the NC1 domains of triple-helical protomers. **(Bottom left)** A space-filling model of the NC1 hexamer quaternary structure shows the location of the sHM crosslinks (white). The sulfilimine bond that constitutes the sHM crosslink connecting the side chains of Met⁹³ and Hyl²¹¹ is shown for the $\alpha 1\text{NC1}$ - $\alpha 1\text{NC1}$ dimer (yellow). The same bond connects Met⁹³ and Hyl²¹¹ in the $\alpha 2\text{NC1}$ - $\alpha 2\text{NC1}$ dimer (magenta).



Although a sulfilimine bond has not been reported in any native biomolecule, they occur in other molecules (23). They are the nitrogen analog of sulfoxides, S(IV), and are also known as sulfur-nitrogen ylides. Most sulfilimines that have been reported are stabilized by strong electron-withdrawing groups on the nitrogen end of the sulfilimine linkage (24). However, cyclic sulfilimines derived from the oxidation of methionine analogs by I_2 have been characterized in detail as their isothiazolidinium salts after protonation of the nitrogen (25, 26). These studies showed direct bonding of the nitrogen and sulfur atoms and a new stereogenic center at the sulfur atom, consistent with the two methyl resonances seen by NMR. In the case of collagen IV, sulfilimine crosslinks are likely to be formed through a two-electron, heteroatom transfer class oxidation (27). For example, the Met⁹³ sulfide could react with a chemical or enzymatic oxidant to form a transient, sulfur(IV) sulfonium intermediate, which is captured by the Hyl²¹¹ or Lys²¹¹ amine to form the sulfilimine. The latter S_N2-like step entails inversion at sulfur because the S-X bond breaks heterolytically and is analogous to the known oxidation of free methionine to dehydromethionine mediated by iodine (26). This sulfilimine linkage may not occur only in collagen IV but in other proteins as well.

Sulfilimines are reduced by thiols to yield the parent amine and thioether groups (23, 24). Thus, the susceptibility of the crosslinked Tp peptide to dithiothreitol (DTT) reduction was evaluated (Fig. 2B). Partial reduction was achieved with 100 mM DTT at room temperature and complete reduction at 80°C at pH = 7.8. Mass spectrometry analyses revealed that DTT breaks the crosslink with the concomitant generation of the T-3599 and T-1412 peptides with complete recovery of both Met⁹³ and Hyl²¹¹, respectively (Fig. 2, A and B). The susceptibility of the crosslink to reduction is comparable to that of disulfide bonds of insulin (fig. S6). Because the crosslinked Tp peptides do not contain cysteines (Fig. 2A), these results provide further support for the existence of a sulfilimine bond between Hyl²¹¹ and Met⁹³.

The location of the sulfilimine linkage within the $\alpha 1(\alpha 2)\alpha 1$ collagen IV network is shown in Fig. 3. Up to six sulfilimine bonds fasten the interface of the trimeric NC1 domains of two adjoining protomers, reinforcing the quaternary structure of the networks. Furthermore, the sulfilimine bond also occurs in the $\alpha 3(\alpha 4)\alpha 5$ collagen IV network (fig. S7) because fragmentation pattern of its crosslinked tryptic peptides (11) is identical to that of the $\alpha 1(\alpha 2)\alpha 1$ network described herein.

The sulfilimine bond likely occurs in diverse metazoan species. NC1 dimer subunits, a signature structural feature indicative of crosslinks,

have been identified in collagenase digests of basement membranes including those of human (28), bovine (28), dog (29), and mouse (28). Furthermore, a phylogenetic analysis of the Lys²¹¹ and Met⁹³ residues, based on a multiple sequence alignment of the NC1 domain across the metazoan phylum [Fig. 4 and (30)], revealed that the sulfilimine bond may occur in many metazoans, except in hydra, flatworm, sponge, and placozoa. A further comparison of the sequence motif [X-K-A, S, or G (31)] that confers hydroxylation of lysyl residues by lysyl hydroxylase (32) occurs in the NC1 domains of all metazoa except hydra, sponge, and placozoa. The motif is also absent in the $\alpha 4$ NC1 domain of human, mouse, bovine, and chick, which in the case of bovine Lys²¹¹ does not undergo hydroxylation and leads to the formation of sKM crosslink (11). In one species of the phylum cnidaria, *Nematostella vectensis*, Met⁹³ and Lys²¹¹ and the hydroxylation motif of Lys are conserved (Fig. 4), suggesting that the sHM crosslink appeared at the time of the divergence of sponge and cnidaria, an apparent evolutionary adaptation that arose in response to mechanical stress on organisms.

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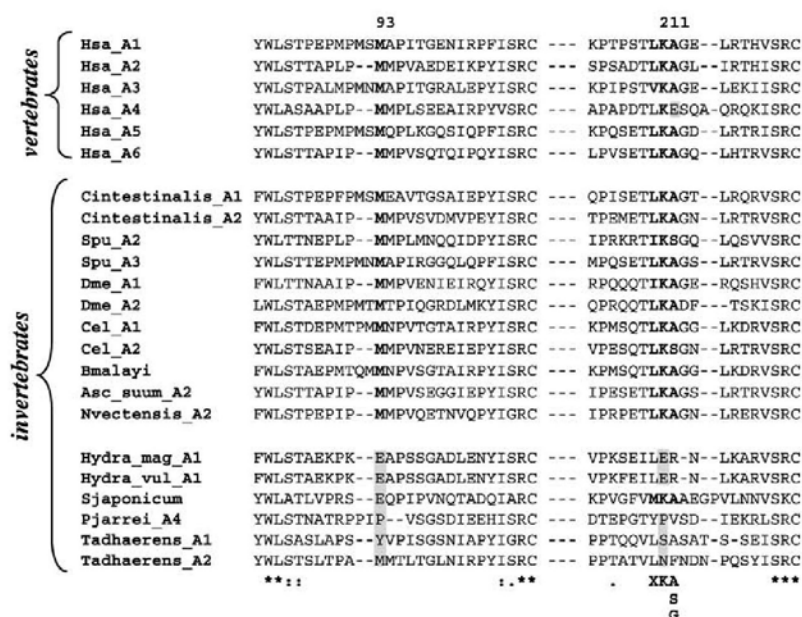


Fig. 4. Multiple sequence alignment of collagen IV NC1 domain sequences encompassing Met⁹³ and Lys²¹¹ (31). Met⁹³ and Lys²¹¹ are shown in bold. Conserved amino acid residues are indicated with an asterisk. Semi-conserved residues are indicated with a colon. The hydroxylation motif for lysyl hydroxylase, X-K-A (A or S or G) is shown at the bottom of the alignment. Abbreviations are as follows: *Homo sapiens* (Hsa) A1, NP_001836.2; A2, NM_001846.2; A3, ABX71213.1; A4, X81053.1; A5, ABW24668.1; and A6, AL136080.6. *Ciona intestinalis* (Cintestinalis) A1, XP_2120982.1; and A2, XP_2119477.1. *Strongylocentrotus purpuratus* (Spu) A2, NP_999676.1; and A3, NP_999631.1. *Drosophila melanogaster* (Dme) A1, AAB28404.1; and A2, AAB64082.1. *Caenorhabditis elegans* (Cel) A1, AAB59179.1; and A2, AAA27989.1. *Brugia malayi* (Bmalayi) XP_1902932.1. *Ascaris Suum* (Asc_suum) AAA18014.1. *Nematostella vectensis* (Nvectensis) XP_1626265.1. *Hydra magnipapillata* (Hydra_mag) XP_2157001.1. *Hydra vulgaris* (Hydra_vul) AAG40729.1. *Schistosoma japonicum* (Sjaponicum) AAX25734.2. *Trichoplax adhaerens* (Tadhaerens) A1, EDV21329.1; and A2, EDV21231.1. The alignments were generated with ClustalW, www.ebi.ac.uk/Tools/clustalw2/index.html (33).

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31. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; X, any amino acid; and Y, Tyr.
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34. We thank D. Liebler for facilitating the initial contact with T. D. Veenstra from SAIC-Frederick, Incorporated, and C. Bolm (Aachen University, Germany) for helpful discussions. The technical assistance of P. Todd and M. Rafi is greatly appreciated. We would also like to thank S. Hill and H. McDonald, from the Proteomics Laboratory at the Mass Spectrometry Research Center of Vanderbilt University, for their assistance with the Orbitrap-LTQ (Thermo Electron, San Jose, CA) instrument. This work was supported in part by NIH grants DK065123 and DK18381 (to B.G.H.), DC007416 (C.R.S.), and GM059380 (P.E.D.); the W. M. Keck Foundation (K.B.S.); and the Skaggs Institute for Chemical Biology (K.B.S.). R.V. and

B.G.H. have filed a provisional patent (1 June 2009) on the potential enzyme system that catalyses the sulfilimine bond formation and that may be involved in pathophysiological processes.

Supporting Online Material

www.sciencemag.org/cgi/content/full/325/5945/1230/DC1
Materials and Methods
Figs. S1 to S7

26 May 2009; accepted 13 July 2009
10.1126/science.1176811

Reassessing the Source of Long-Period Comets

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We present numerical simulations to model the production of observable long-period comets (LPCs) from the Oort Cloud, a vast reservoir of icy bodies surrounding the Sun. We show that inner Oort Cloud objects can penetrate Jupiter's orbit via a largely unexplored dynamical pathway, and they are an important, if not the dominant, source of known LPCs. We use this LPC production to place observationally motivated constraints on the population and mass of the inner Oort Cloud, which are consistent with giant planet formation theory. These constraints indicate that only one comet shower producing late Eocene bombardment levels has likely occurred since the Cambrian Explosion, making these phenomena an improbable cause of additional extinction events.

Because of their large distances from the Sun, Oort Cloud bodies undergo orbital evolution driven by gravitational perturbations from passing stars and the galactic tide (1, 2). During this process, semimajor axes (a) remain nearly constant while perihelia (closest approach distances to the Sun, or q) evolve under these perturbations. Although most Oort Cloud bodies have perihelia far outside the planetary region of the Solar System, a tiny fraction are continually injected into planet-crossing orbits (3). Once there, they receive energy kicks from planetary perturbations during each perihelion passage, causing the semimajor axes to change at random. The magnitude of these kicks increases greatly near Jupiter and Saturn, and in the inner 10 to 15 astronomical units (AU) of the Solar System the typical planetary energy kick is greater than the gravitational binding energy of Oort Cloud orbits (4). Thus, inward perihelion drift of long-period comets (LPCs) is halted inside ~10 to 15 AU because they are either immediately ejected to interstellar space or perturbed onto low- a (and therefore fixed- q) orbits before eventually being ejected. This effect, known as the Jupiter-Saturn barrier, prevents many Oort Cloud bodies from passing near Earth.

To reach an observable orbit ($q < 5$ AU), an Oort Cloud body's perihelion must therefore decrease from outside 10 to 15 AU to inside 5 AU in less than one orbital period. Bodies with $a < \sim 20,000$ AU are less sensitive to galactic

perturbations, and, except during rare close stellar passages causing comet showers, their perihelia evolve too slowly to reach observable orbits before ejection. For this reason, the Jupiter-Saturn barrier has traditionally been thought to prevent bodies in the inner 20,000 AU of the Oort Cloud from evolving to currently observable LPCs (2), and this has been the motivation for dividing the Oort Cloud into the inner (unobservable) cloud with $a < 20,000$ AU and outer (observable) cloud with $a > 20,000$ AU. The edge of the Jupiter-Saturn barrier is not abrupt, however, and recent modeling of scattered disk orbits indicates that repeated smaller planetary energy kicks near the barrier edge can inflate low- a orbits to $a > 20,000$ AU (5).

Here, we present numerical simulations of the production of observable LPCs (6), and we find that many observable LPCs ultimately originate from the inner Oort Cloud (Fig. 1). In the case shown here, an inner Oort Cloud body with an initial semimajor axis of 6000 AU had a perihelion slowly evolving Sunward under the influence of the galactic tide. When the perihelion was beyond ~18 AU, the semimajor axis was nearly unaltered by the relatively weak perturbations of Uranus and Neptune. Between 18 and 14 AU, however, a was rapidly inflated to ~30,000 AU. With such a large semimajor axis, the perihelion then decreased by 13 AU during the next orbital period, circumventing the Jupiter-Saturn barrier and evolving to an observable LPC. About 85% of inner Oort Cloud bodies evolving to observable LPCs followed an evolution similar to that shown in Fig. 1. Details of other known minor LPC production pathways that are less direct and efficient (7) can be found in the supporting online material (SOM) text.

Although LPC production has been modeled previously, a substantial inner Oort Cloud contribution has hitherto gone unnoticed. In some models, this is because planetary perturbations are not included (8, 9), leaving no mechanism to inflate a near the Jupiter-Saturn barrier. However, some works include the full gravity of the giant planets (3, 10). In these instances, orbital elements of incoming LPCs are sampled when they have already attained $q < 5$ AU, obscuring any prior a evolution. We performed a similar orbit sampling of dynamically new LPCs (LPCs entering the observable region for the first time) (Fig. 2A). The resulting a distribution hides the inner Oort Cloud's contribution.

To assess the inner Oort Cloud's LPC production, we instead considered the initial semimajor axes from which LPCs evolved in our simulation (Fig. 2A). This distribution indicates that just over half of all dynamically new LPCs come from the inner Oort Cloud, which is a conservative estimate because we used an Oort Cloud model with a low inner-to-outer population ratio of 1.5:1 (see SOM text for a treatment of alternative models). Below $a \sim 5000$ AU, LPC production fell dramatically because of the nonisotropic nature of the innermost part of our Oort Cloud model (fewer orbits had decreasing perihelia in this region). However, even additional simulations with a more-isotropized

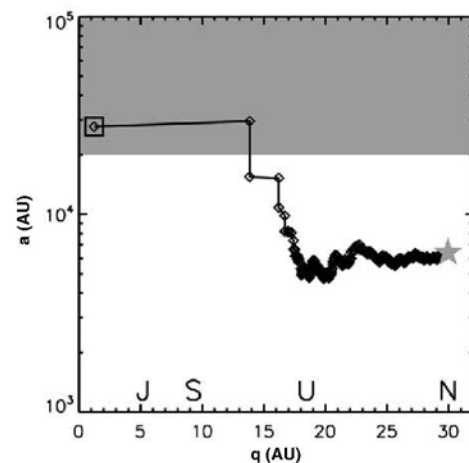


Fig. 1. An example of the typical evolution from an inner Oort Cloud object into an observable LPC. Orbital elements are sampled each time the object crosses the $r = 35$ AU boundary (twice per orbit). The star data point marks the start of the evolution and the square marks the end. The shaded area indicates the a range of the outer Oort Cloud, and the perihelia of the giant planets are noted with their initials.

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inner Oort Cloud (11) indicate greatly suppressed LPC production below $a \sim 3000$ AU. Thus, many bodies may reside inside ~ 3000 AU without producing observable LPCs (SOM text), but any population with $a \gg 3000$ AU will be reflected and constrained by the observed LPC population.

Because detecting LPCs outside the Jupiter-Saturn barrier is difficult, very few candidate inner Oort Cloud objects are currently known (12, 13).

However, as shown in Fig. 2A, our simulations predict that a substantial fraction of the hundreds of cataloged LPCs come from the inner Oort Cloud. By assuming the inner Oort Cloud generates the entire observed LPC flux, we can estimate an upper limit on the number of cometary bodies with $a \gg 3000$ AU. Although the measured flux of LPCs is uncertain, even the highest flux values quoted in the literature (14) imply an inner Oort

Cloud population below $\sim 10^{12}$, a factor of 2 to 3 greater than previously accepted estimates of the outer Oort Cloud population (15).

Efficient LPC production from inner Oort Cloud orbits may also help resolve a possible inconsistency between the Oort Cloud mass and planet formation. Because the outer Oort Cloud traps only 1 to 2% of planetesimals scattered by giant planets (11), outer Oort Cloud population estimates imply an unreasonably massive primordial solar nebula for many assumed LPC population models (16). Although LPC population uncertainties may explain this discrepancy (SOM text), inner Oort Cloud LPC production offers an alternative explanation. This is because the inner Oort Cloud's trapping efficiency substantially exceeds that of the outer cloud if the Sun formed in a star cluster (11, 17). We predicted a new range of solar nebula masses as a function of inner Oort Cloud trapping efficiency (Fig. 2B), assuming all known LPCs come from this region. Higher trapping efficiencies (5 to 10%) provided by a solar birth cluster imply a solar nebula mass range (20 to 100 times the mass of Earth in solids) compatible with giant planet formation theory (18, 19). This raises the possibility that known LPCs can be used to discriminate between solar birth environments (6).

With an upper estimate on the population between $3000 \text{ AU} < a < 20,000 \text{ AU}$, we can now also determine the maximum number of LPCs delivered during comet showers because this region supplies the excess LPCs absent during nonshower periods (20). From numerical experiments (6) of individual stellar encounters (an example of which is shown in Fig. 3A), we find that the number of comets injected into Earth-crossing orbits scales with the Sun's velocity change resulting from the encounter (Fig. 3B). With use of a stellar population model for the solar neighborhood (9), we transformed this into a prediction of comet shower strength versus shower frequency (Fig. 3C). By attributing the observed Earth-crossing LPC flux estimate [two dynamically new LPCs per year (8)] to just the inner Oort Cloud, we predicted a maximum impact number (21) expected for a given comet shower (Fig. 3C).

Spikes in the flux of Earth-impacting LPCs during comet showers have previously been suggested as a possible cause of mass extinctions seen in the fossil record (22). With three nearly simultaneous major impacts accompanied by a 2.5-million-year (My) ^3He spike, a comet shower is a particularly appealing explanation for the late Eocene extinction (23). On the basis of our upper estimate of the inner Oort Cloud population, it is expected that the most powerful comet shower in the past 500 My yielded just two to three impacting comets greater than ~ 2 km in diameter (Fig. 3C). If the late Eocene episode was caused by a comet shower, it was likely the most powerful shower since the Cambrian Explosion, implying that comet showers are unlikely to account for other observed extinction events. Semimajor axis distributions much more extreme than our chosen Oort Cloud model can raise the shower intensity

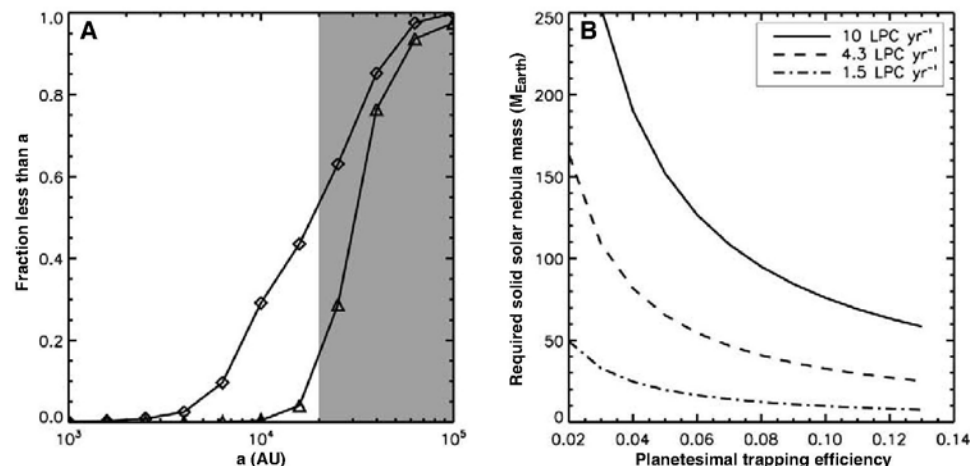


Fig. 2. (A) Cumulative semimajor axis distributions for dynamically new LPCs. The two distributions are the initial ($t = 0$) values (diamonds) and when q first drops below 5 AU (triangles). (B) The required solar nebula mass in solids [adopting an average LPC mass of 4×10^{16} g as a fiducial value (24)] as a function of inner Oort Cloud trapping efficiency for an assumed dynamically new LPC flux. The different curves correspond to flux values of 10 (14), 4.3 (25), and 1.5 (26) dynamically new LPCs per year.

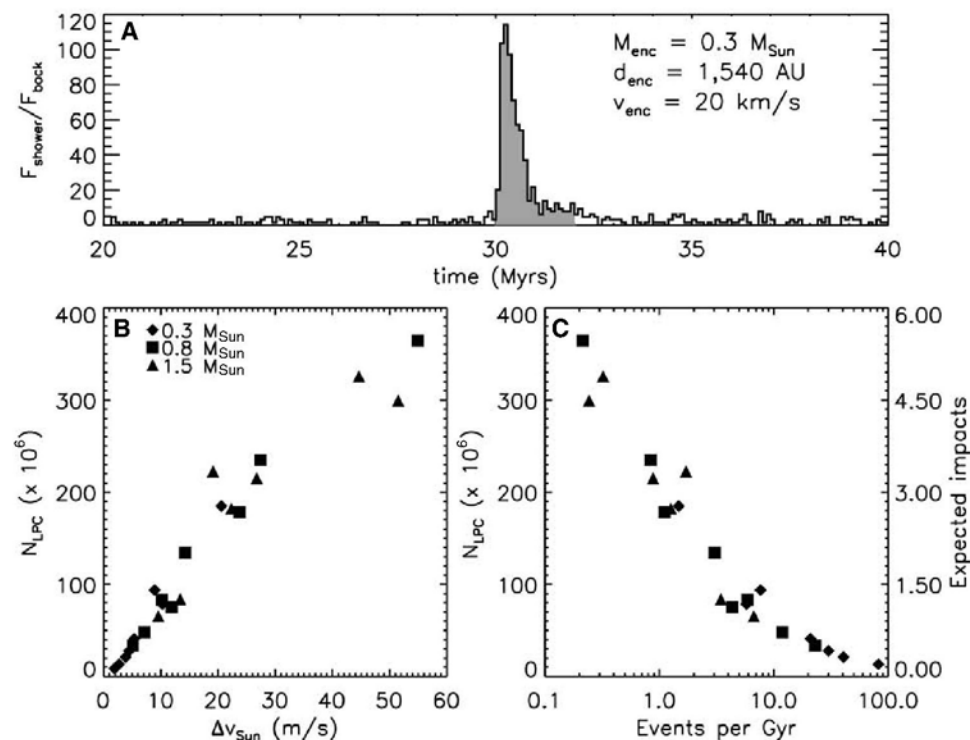


Fig. 3. (A) Flux of dynamically new LPCs (in units of the average background level) versus time during a comet shower at $t = 30$ My. To quantify comet shower strength, all dynamically new LPCs in the 2 My after the stellar passage are counted (shaded region). (B) The number of Earth-crossing LPCs delivered during comet showers versus the solar velocity change from the stellar encounter (assuming equal scaling of observable and Earth-crossing LPCs). The different symbols refer to different stellar encounter masses (M_{enc}). Encounter distances (d_{enc}) ranged from 1300 AU to 7000 AU, and velocities (v_{enc}) were between 20 and 40 km/s. (C) The number of Earth-crossing comets delivered in comet showers versus the expected shower frequency at that strength. On the right y axis, the expected number of terrestrial impacts with a comet greater than ~ 2 km in diameter is shown.

by a factor of ~ 3 , but solar nebula mass requirements to form these configurations make them physically implausible (see SOM text for details).

Although known LPCs constrain the total population beyond $a \sim 3000$ AU, they offer little information about the relative inner and outer Oort Cloud populations because LPC production obscures orbital histories. However, inner cloud LPC production predicts the generation of $a > 20,000$ AU orbits near Jupiter and Saturn. In contrast, the outer Oort Cloud's $a > 20,000$ AU population will decrease after passing through this region. Thus, a comparison of original and future semimajor axes for a large LPC sample near $q \sim 10$ AU (analogous to that done for currently known LPCs) could provide an opportunity to distinguish between production mechanisms.

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27. This research was funded by a NASA Earth and Space Science Fellowship and an NSF grant (AST-0709191). Our computing was performed by using Purdue TeraGrid computing facilities managed with Condor scheduling software (www.cs.wisc.edu/condor). A. Morbidelli and two anonymous reviewers provided helpful comments that greatly improved our work.

Supporting Online Material

www.sciencemag.org/cgi/content/full/1172676/DC1

Materials and Methods

SOM Text

Figs. S1 to S6

References

23 February 2009; accepted 10 July 2009

Published online 30 July 2009;

10.1126/science.1172676

Include this information when citing this paper.

Recent Warming Reverses Long-Term Arctic Cooling

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The temperature history of the first millennium C.E. is sparsely documented, especially in the Arctic. We present a synthesis of decadal resolved proxy temperature records from poleward of 60°N covering the past 2000 years, which indicates that a pervasive cooling in progress 2000 years ago continued through the Middle Ages and into the Little Ice Age. A 2000-year transient climate simulation with the Community Climate System Model shows the same temperature sensitivity to changes in insolation as does our proxy reconstruction, supporting the inference that this long-term trend was caused by the steady orbitally driven reduction in summer insolation. The cooling trend was reversed during the 20th century, with four of the five warmest decades of our 2000-year-long reconstruction occurring between 1950 and 2000.

As awareness of the recent rapid changes in the Arctic grows (1), so too does the need for a longer-term perspective on these changes. This study places the warming of the instrumental period against the backdrop of the past 2000 years, well beyond the 400-year scope of the last Arctic-wide synthesis of high-resolution paleoclimate data (2). Our synthesis is based on a new compilation of proxy records from Arctic lakes (3),

combined with complementary ice core and tree ring records, to form a new 2000-year-long, decadal resolved paleoclimate reconstruction for the Arctic. Lakes are distributed across the Arctic, and they contain the most accessible proxy records that consistently extend through the late Holocene. The synthesis is restricted to records longer than 1000 years because we aim to explore the long-term pattern of temperature variability at decadal scale. These records extend beyond the most recent (pre-industrial) major climate perturbation—the Little Ice Age—when most of the Arctic experienced the coldest sustained temperatures of the past 8000 years (4). In some locations, warm intervals before the Little Ice Age have been recognized during the early part of the period from 2000 to 1000 years ago, as well as during the Middle Ages (5). The spatial coherence of the warming during these intervals is not yet clear (6), but this is critical for understanding the underlying causes of change. Climate change is amplified in the Arctic (7), and warming during these historical intervals might

be more reliably detected where the temperature change exceeds the sensitivity limits of the proxies.

We compiled available proxy climate records that (i) were located north of 60°N latitude, (ii) extended back at least 1000 years, (iii) were resolved at an annual to decadal level, and (iv) were published with publicly available data (8) (table S1) (www.ncdc.noaa.gov/paleo/pubs/kaufman2009). We focused on terrestrial records because the dating resolution for most marine cores is too low to reconstruct decadal-scale variability. Our compilation includes 23 sites where lake sediment, glacier ice, and tree rings have been used as paleoclimatic archives (Fig. 1). The observed summer [June, July, and August (JJA)] temperature in the grids represented by the 23 proxy sites (Fig. 1) closely tracks the temperature for all of the land area north of 60° latitude, indicating that our proxy network accurately represents the Arctic-wide mean (8) (fig. S1). Twelve of the records are based on sedimentological and biological indicators from lakes, mainly varve properties and productivity indicators. These proxies reflect changes in summer temperatures, a primary control on physical and biological processes in lakes at high latitudes (9). Seven of the records are from glacier ice, mostly from Greenland. These rely on oxygen isotopes, which reflect a combination of the temperature and moisture transport history of the snow that accumulated on the ice sheet throughout the year (10). Four of the records are based on the width of tree rings, which have been interpreted primarily as a proxy for warm-season temperature, and were processed using the regional curve standardization procedure to help preserve the long-term variability (11). The chronologies for nearly all records are based on annual layer counting, and most authors report accuracies to within $\pm 2\%$. Some of the lake records used radioisotopes to model downcore trends in sedimentation rate, with uncertainties within $\pm 10\%$ for the past 2000 years.

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Our “composite-plus-scale” synthesis (12) focuses on 10-year-mean temperatures to accommodate records that are not annually resolved and to minimize the effect of minor age uncertainties. In addition, the coherence of the regional climate signal is greater on decadal time scales than inter-annually (13). Each record was subdivided into 200 10-year intervals, and the average series were standardized to a mean of zero and unit variance relative to the 820-year period (980 to 1800) common to all records (table S2). The records were then composited by averaging the standardized series without weighting. The 10-year-mean proxy values from the 19 records that extend into the late 20th century (Fig. 2) were used to generate a least-squares linear regression that scales the proxy data to the Arctic-wide summer temperature ($r^2 = 0.79$, $P < 0.01$) (8). We used the spatially averaged summer temperature for all land area north of 60° latitude from the CRUTEM3 data series (14).

Among the most striking features of our composite temperature reconstruction is a cooling from 1 C.E. to 1900 C.E. (Fig. 3). The cooling trend is especially clear in records from ice and lakes (Fig. 3A). For trees, only three records extend back before 720, which is not enough to determine a reliable trend. The cooling trend is based on the 17 records that extend back two millennia (Fig. 3B). The other six records that extend back to at least 980 track the longer-term records for the period of overlap ($r^2 = 0.23$, $P < 0.01$). Least-squares linear regression yields a cooling trend of $-0.22^\circ \pm 0.06^\circ\text{C}$ per 1000 years (8) (Fig. 3C).

Principal components (PC) analysis was used to extract the dominant mode of variability from the 15 standardized records that extend from 1 C.E. to 1900 C.E. (table S1). The leading mode explains 17% of the variance of all records, and its time series is similar to the simple composite of the records for this period ($r^2 = 0.84$, $P < 0.01$). All records are positively correlated with PC1, indicating that the trends are predominantly of the same sign over the 1900-year period.

Because the unweighted composite and the PC of the records at 10-year intervals could be skewed by extreme values from a few records, we generated an alternative composite record using the approach of Osborn and Briffa (15). For this procedure, the number of available records for each 10-year interval with a proxy value that exceeded either +1 or -1 SD was tallied, and the difference in the proportion of records that exceeded these thresholds was calculated by subtraction. The resulting time series shows a monotonic decrease similar to the mean composite and to the PC values (Fig. 3E).

The millennial-scale cooling trend is consistent with other proxy evidence showing that summer temperatures across the Arctic reached their maximum during the first half of the present interglaciation (between about 10,000 and 6000 years ago), then cooled into neoglaciation (4). For example, summer melt became less frequent on ice caps in northeastern Canada (16), the tree line retreated southward across northern Eurasia (17), herbaceous tundra expanded in Fennoscandia (18), and gla-

ciers expanded in mountains across the Arctic (19). Proxy data from the western hemisphere of the Arctic indicate that summer temperatures were $1.6^\circ \pm 0.8^\circ\text{C}$ higher during the Holocene thermal maximum (HTM) than the average of the 20th century (20). The timing of the HTM transgressed from west to east across the North American Arctic but generally peaked around 7500 years ago, suggesting a post-HTM cooling rate between -0.11° and -0.32°C per 1000 years. This compares well with the average cooling rate of $-0.22^\circ \pm 0.06^\circ\text{C}$ per 1000 years derived here for our 2000-year time series of Arctic summer temperature (Fig. 3C).

Fig. 1. Locations of the proxy climate records included in the synthesis. Map colors indicate trends in summer (JJA) temperature between 1958 and 2000 from the ERA-40 data series (34). Large and small symbols indicate records that extend back to 2000 years ago (2 ka) and to at least 1000 years ago, respectively. Site numbers are keyed to table S1.

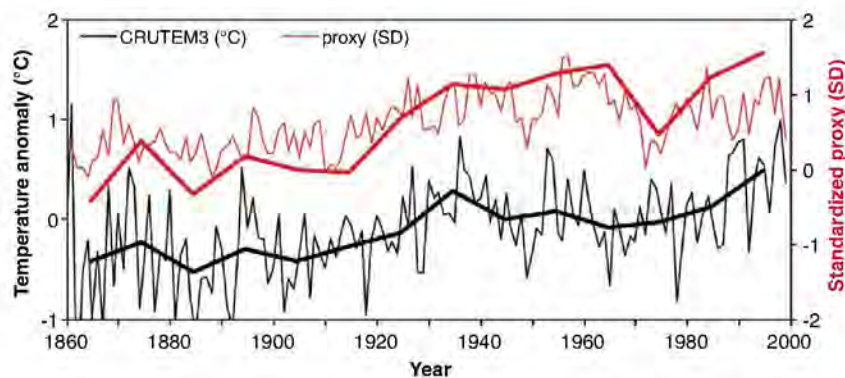
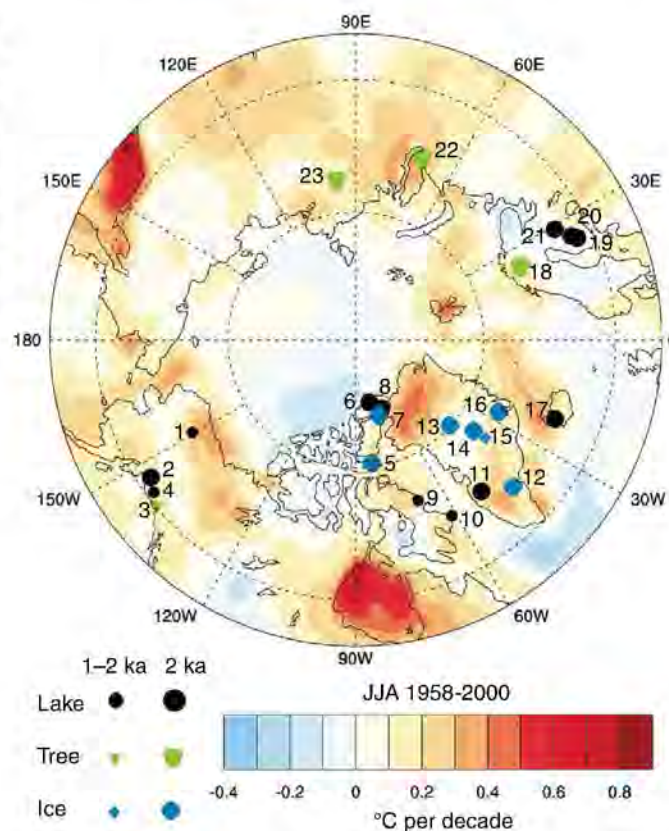


Fig. 2. Comparison between Arctic-wide mean summer (JJA) temperature anomalies relative to the period 1961–1990 based on the CRUTEM3 data series (14) and mean standardized proxy values (SD units). The annual proxy values (narrow red line) are averaged from the 10 sites with annually resolved time series that extend into the late 20th century, whereas the 10-year-mean proxy values (bold red line) are based on all 19 sites with records that extend through the 20th century. Ten-year means (bold lines) were used to derive a regression equation to scale our new proxy record to summer temperature (8).

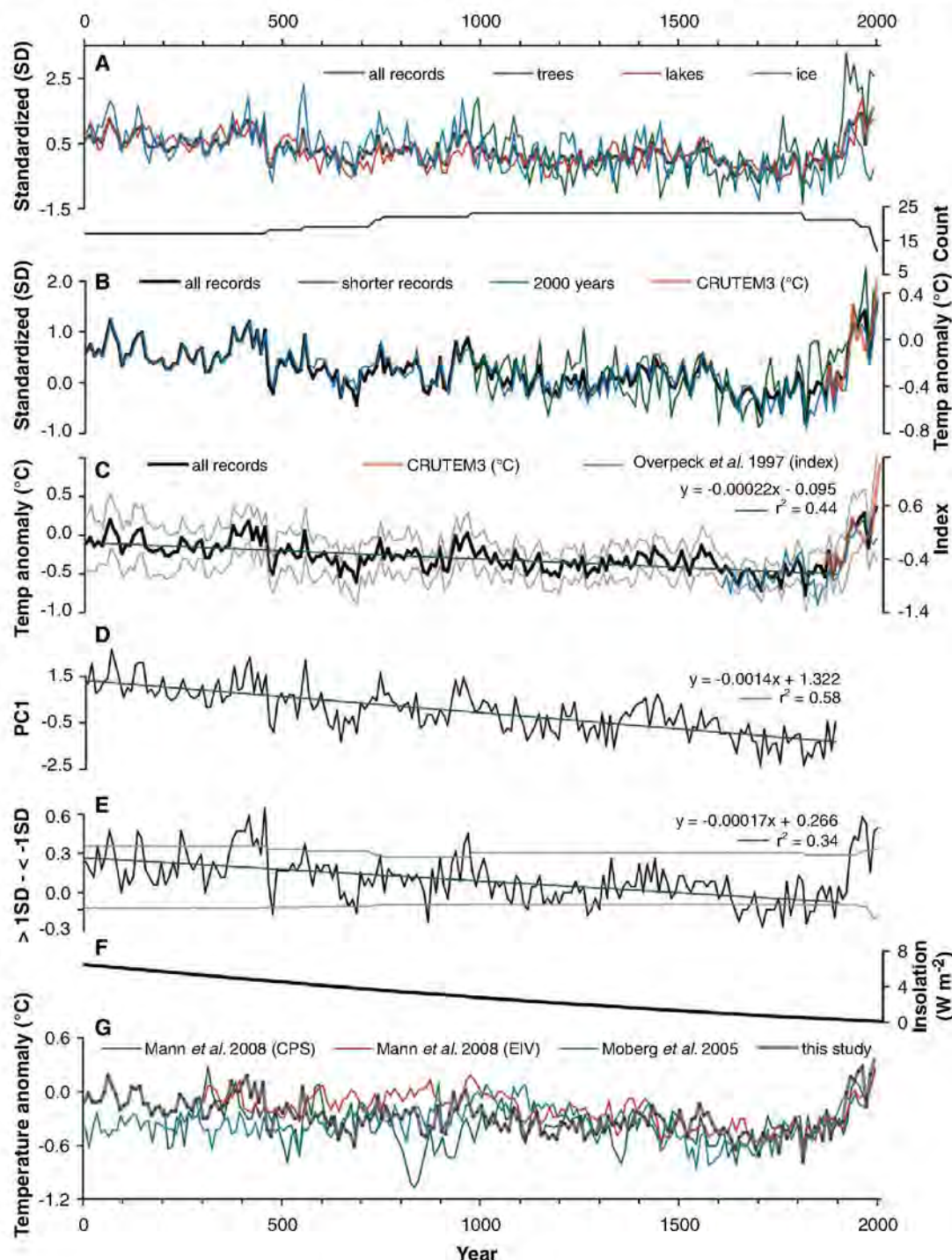
Assuming that the overall cooling since the HTM was ultimately caused by the decrease in summer insolation (19), it seems likely that this insolation anomaly of +1.4% relative to the present at 65°N was amplified by climate feedbacks (22). The strongest positive feedbacks in the Arctic are related to changes in terrestrial snow and sea-ice cover, which (like our climate proxies themselves) are sensitive to warm-season temperatures and respond rapidly to summer insolation anomalies. As sea ice expanded after the HTM (23), both the amount of solar energy absorbed by the ocean surface waters and the transfer of heat from the surface water to the atmosphere were diminished. The expansion of tundra into areas formerly cov-

ered by shrubs or forest may have further contributed to the terrestrial snow and land-cover albedo feedback (24).

These sea-ice, snow, and terrestrial feedbacks were represented in a transient, mid-late Holocene simulation with the Community Climate System Model (CCSM3) (8, 25). For the period from 5600 to 3600 years ago, orbital forcing was the strongest time-varying forcing of this simulation. Results from this simulation show that the relation between summer insolation and temperature in the model is the same as for the proxy reconstruction, thereby supporting the connection between the Arctic summer cooling trend and the orbitally driven reduction in summer insolation (Fig. 4).

In contrast to our regional reconstruction, recently published syntheses of the Northern Hemisphere average temperature do not show an overall 1900-year-long cooling trend (26, 27) (Fig. 3G). Rather, they are dominated by centennial-scale fluctuations, with little indication that the first 1000 years C.E. was warmer than the millennium that followed. The centennial-scale anomalies around the long-term trend in our high-latitude reconstruction appear to correspond with the temporal structure from the Northern Hemisphere as a whole. The period from about 450 to 700 was generally cooler than the linear trend, and the period from 900 to 1050 tended to be warmer. In good agreement with other reconstructions (28), the coldest interval

Fig. 3. (A and B) Composite of 23 high-resolution proxy climate records from the Arctic. Values are 10-year means standardized relative to the reference period of 980 to 1800. (A) Records subdivided by source of proxy information: trees, ice, and lakes, with the running count of records. (B) Records subdivided by those that extend 2000 years ($n = 17$) versus shorter records ($n = 6$), along with the 10-year-mean Arctic-wide summer temperature through 2000 from the CRUTEM3 data series (14) (red line). (C) Mean of all records transformed to summer temperature anomaly relative to the 1961–1990 reference period, with first-order linear trend for all records through 1900 (green line), the 400-year-long Arctic-wide temperature index of Overpeck *et al.* (2) (blue curve; 10-year means), and the 10-year-mean Arctic temperature through 2008 (red line). Gray lines encompass ± 2 standard errors of the proxy values as evaluated for each 10-year interval. (D) Time series of PC1 based on the 15 records that extend from 1 C.E. to 1900 C.E., showing a strong first-order trend. (E) Difference in the fractional proportion of records that exceed ± 1 SD for each 10-year interval. Gray lines are 95th percentile of distributions determined by 10,000 Monte Carlo realizations of shifting the time series randomly in time [as in (15)]. (F) Change in summer (JJA) insolation at 65°N latitude relative to the 20th century (21). (G) Northern Hemisphere average proxy temperature anomalies (10-year means) reconstructed by Mann *et al.* (26) on the basis of two approaches (CPS, composite plus scale; EIV, error in variables) and by Moberg *et al.* (27). Our Arctic regional reconstruction is overlaid in gray.



occurred between 1600 and 1850. The Arctic record therefore supports the centennial-scale variations documented elsewhere, which can be reasonably explained by solar irradiance and volcanic forcing (29).

Strong warming in the 20th century contrasts sharply with the preceding cooling trend. An Arctic summer temperature of -0.5°C (relative to the period 1961–1990) might have been expected by the mid-20th century on the basis of a simple forward projection of the linear trend in the proxy data for the period from 1 C.E. to 1900 C.E. (Fig. 3C). Instead, our reconstruction indicates that temperatures increased to $+0.2^{\circ}\text{C}$ by 1950. This shift correlates with the rise in global average temperature, which coincided with the onset of major anthropogenic changes in global atmospheric composition, the absence of major volcanic eruptions, and changes in solar irradiance (30). During the early 20th century, warming in the Arctic was enhanced relative to the global average, likely reflecting a combination of natural variability (31) and positive

feedbacks that amplified the radiative forcing (7). During the late 20th century, our proxy-inferred summer temperatures were the warmest of the past two millennia, with four of the five warmest decades of our 2000-year-long reconstruction occurring between 1950 and 2000. In recent years, the magnitude of the warming seems to have emerged above the natural variability, consistent with the sharp reduction in summer sea-ice cover (32) and the rapid increase in biological activity in circum-Arctic lakes (9).

The strongest trend in our proxy temperature reconstruction is the millennial-scale cooling of $-0.22^{\circ}\text{C} \pm 0.06^{\circ}\text{C}$ per 1000 years. The cooling corresponds with the slow reduction in summer insolation at high northern latitudes, driven by orbital forcing and enhanced by positive feedbacks that amplified the forcing more strongly than at lower latitudes. Summer insolation correlates with summer temperature in our proxy reconstruction and in a 2000-year climate simulation by CCM3 (Fig. 4). Orbitally driven summer insolation continued to decrease through the 20th century, implying that summer temperatures should have continued to cool. Instead, the shift to higher temperatures during the 20th century reversed the millennial-scale cooling trend. The warming during the 20th century (and first decade of the 21st century) contrasts sharply with the millennial-scale cooling, with the last half-century being the warmest of the past two millennia. Our synthesis, together with the instrumental record, suggests that the most recent 10-year interval (1999–2008) was the warmest of the past 200 decades. Temperatures were about 1.4°C higher than the projected value based on the linear cooling trend and were even more anomalous than previously documented.

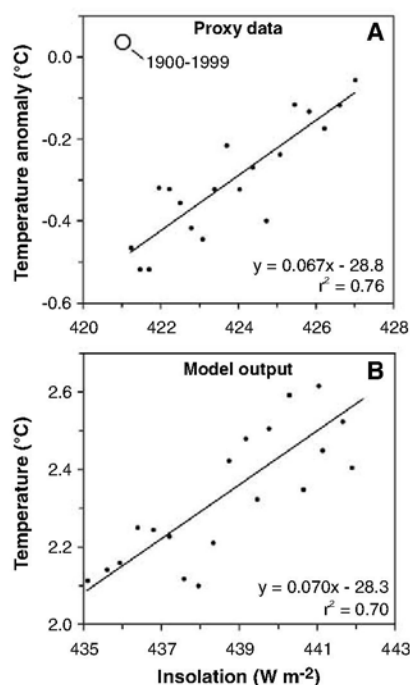


Fig. 4. Sensitivity of Arctic summer (JJA) temperature to orbital forcing, as inferred from (A) our proxy-based reconstruction, and (B) a 2000-year simulation by the Community Climate System Model (CCSM3). For (A), temperatures are 100-year-mean JJA values relative to the 1961–1990 reference period. The 20th century (open circle) is anomalous with respect to the trend defined by the previous 1900 years. The slope of the least-squares regression implies a regional sensitivity of $0.07^{\circ}\text{C} \pm 0.02^{\circ}\text{C}$ per W m^{-2} for the proxy-based estimate. Insolation is average JJA at 65°N from Berger and Loutre (21). For (B), temperatures are from CCSM3-simulated (8) 100-year-mean JJA area-averaged values for land north of 60°N latitude. The model-derived sensitivity is also $0.07^{\circ}\text{C} \pm 0.02^{\circ}\text{C}$ per W m^{-2} . Insolation is average JJA at 65°N from the CCSM3 simulation. Axis scales in (A) and (B) are the same for visual comparison of regression slope; values differ because (B) is based on model conditions for the period from 5600 to 3600 years ago.

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35. Supported by the Arctic System Science Program of NSF [grants ARC-0455043 (D.S.K.), 0454959 (R.S.B.), 0455025 (G.H.M.), 0450938 (J.T.O.), and 0454930 (B.L.O.-B.)]. NCAR is funded by NSF. We thank our collaborators on the ARCS 2k Project (www.arcs.org/synthesis2k) for their contributions, and M. Hughes, K. Kreutz, and reviewers for their input. NOAA Paleoclimatology assisted with data management.

Arctic Lakes 2k project members include the primary contributors to (3): M. Abbott,¹ Y. Axford,² B. Bird,³ H. J. B. Birks,³ A. E. Bjune,⁴ J. Briner,⁵ T. Cook,⁶ M. Chipman,⁷ P. Francus,⁸ K. Gajewski,⁹ A. Geirsdóttir,¹⁰ F. S. Hu,¹¹ B. Kutchko,¹ S. Lamoureux,¹² M. Loso,¹³ G. MacDonald,¹⁴ M. Peros,⁹ D. Porinchu,¹⁵ C. Schiff,¹⁶ H. Seppä,¹⁷ E. Thomas⁵

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Supporting Online Material

www.sciencemag.org/cgi/content/full/325/5945/1236/DC1
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24 March 2009; accepted 22 June 2009
10.1126/science.1173983

Poly(ADP-ribose)–Dependent Regulation of DNA Repair by the Chromatin Remodeling Enzyme ALC1

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Posttranslational modifications play key roles in regulating chromatin plasticity. Although various chromatin-remodeling enzymes have been described that respond to specific histone modifications, little is known about the role of poly[adenosine 5′-diphosphate (ADP)–ribose] in chromatin remodeling. Here, we identify a chromatin-remodeling enzyme, ALC1 (Amplified in Liver Cancer 1, also known as CHD1L), that interacts with poly(ADP-ribose) and catalyzes PARP1-stimulated nucleosome sliding. Our results define ALC1 as a DNA damage–response protein whose role in this process is sustained by its association with known DNA repair factors and its rapid poly(ADP-ribose)–dependent recruitment to DNA damage sites. Furthermore, we show that depletion or overexpression of ALC1 results in sensitivity to DNA-damaging agents. Collectively, these results provide new insights into the mechanisms by which poly(ADP-ribose) regulates DNA repair.

Processes such as transcription, repair, and replication that require efficient DNA recognition are dependent on modulation of chromatin structure (1–3). Chromatin relaxation is a critical event that occurs during DNA repair and is associated with the negatively charged polymer of adenosine 5′-diphosphate (ADP)–ribose (PAR) (4). PAR is synthesized from nicotinamide adenine dinucleotide (NAD⁺) by the poly(ADP-ribose) polymerase protein family (PARPs), of which PARP1 (and to a lesser extent PARP2) respond to DNA strand breaks (5–7). As a consequence of poly(ADP-ribosylation), chromatin adopts a more relaxed structure (8–11), and this is thought to facilitate DNA repair (4, 12–14). However, the molecular mechanism by which PAR modulates chromatin during DNA repair is largely unknown.

One potential mechanism for PAR function is to bind and recruit chromatin modifiers. In a search for DNA repair–associated chromatin factors, we investigated the function of human ALC1 (Amplified in Liver Cancer 1, also known as CHD1L), which contains a helicase domain (Fig. 1A) that is also found in the Snf2 family of adenosine 5′-triphosphate (ATP)–dependent chromatin-remodelers (such as Snf2, ISWI, and CHD1) (15). ATPases of

this family are modular in nature and often combine a helicase domain with motifs that mediate selective recognition of protein modifications. Unlike CHD1, ALC1 does not contain a chromo domain, which can recognize methylated histone tails. Instead, ALC1 contains a macro domain, which is an ADP-ribose/PAR-binding element (16). Thus, ALC1 might possess a PAR-dependent chromatin-remodeling activity and facilitate DNA repair reactions within a chromatin context.

To assess this, we determined whether ALC1 binds PAR *in vitro*. We constructed a number of ALC1 domain deletions, as well as the putative ATPase dead mutant K77R, and the macro-domain mutant D732A, which is predicted to affect PAR binding (Fig. 1A) (16–19). Purified recombinant FLAG-tagged wild-type (WT) ALC1 and the various mutated ALC1 derivatives (fig. S1A) were dotted onto a nitrocellulose membrane, and their ability to bind ³²P-radiolabeled PAR was measured. Aprataxin PNK-like factor (APLF), which binds PAR via an unrelated PAR-binding Zn-finger, was used as a positive control (20). PAR binding was detected with the macro domain-containing ALC1 proteins (WT, K77R, and C1) but not with the N-terminal helicase domain fragments N1 and N2 or with the macro-domain mutant D732A (Fig. 1B). We also demonstrated PAR binding in cells by means of immunoprecipitation of the FLAG-tagged ALC1 proteins from transiently transfected 293T cells. Endogenous PAR was immunoprecipitated with the macro domain-containing proteins but not with the D723A mutant or the helicase domain fragments N1 and N2 (Fig. 1C). Most of the immunoprecipitated PAR was associated with PARP1 and histones; we observed signals on the blot of antibody to PAR whose molecular weights corresponded to these proteins (Fig. 1C). We also detected increased levels of endogenous PAR in the extracts of cells that expressed the macro-domain proteins (WT, K77R, and C1) (Fig. 1C, inputs).

Possibly, overexpression of these PAR-binding proteins restricted accessibility of PAR to be degraded by the catabolic enzyme poly(ADP-ribose) glycohydrolase (PARG), giving rise to the observed effect. The ALC1 immunoprecipitates also revealed interactions of the macro-domain proteins with PARP1 and core nucleosome components (Fig. 1C). The interaction with PARP1 was severely reduced with the D723A mutant.

We next tested ALC1 for nucleosome-stimulated ATPase activity. ALC1 displayed only weak ATPase activity on its own, but the activity was stimulated modestly by the addition of DNA and more markedly by the addition of nucleosomes (Fig. 1D). This enhanced activity was, however, less pronounced with nucleosomes containing histone H4 mutated at residues 16 to 19 [H4(16–19)A], demonstrating that the ALC1 ATPase activity required the histone H4 N-terminal tail (fig. S1D). A single amino acid change in the ATP-binding Walker A motif (K77R) abolished the ATPase activity of ALC1 (Fig. 1D).

To establish ALC1 as a bona fide chromatin-remodeling enzyme, we tested its ability to reposition nucleosomes. Native-gel analyses showed that WT ALC1 and the macro-domain mutant D732A, but not the ALC1 ATPase dead mutant K77R, catalyzed nucleosome sliding in an ATP-dependent manner (Fig. 1E). The ability of ALC1 to reposition nucleosomes was histone H4 tail-dependent; it was not observed with the mutant nucleosome H4(16–19)A (fig. S1F). The dependence on this epitope within the H4 tail has been observed with other remodeling enzymes (21, 22) and provides strong evidence that nucleosomes are the relevant substrate for ALC1.

Given that ALC1 interacts with PAR and PARP1, we investigated the effect of PARP1 action on the activities of ALC1. PARP1 stimulated the ATPase activity of ALC1 approximately fourfold, depending on the assay conditions (Fig. 1F and fig. S1H). Stimulation was not observed in the absence of NAD⁺ or DNA (Fig. 1F and fig. S1, D and H) and was abolished by PARP inhibitor treatment (Fig. 1F), indicating that poly(ADP-ribosylation) was required for ALC1 stimulation. Preincubation of PARP1 with nucleosomes and NAD⁺, followed by the addition of PARP inhibitor, was sufficient for ALC1 stimulation (Fig. 1F), suggesting that the stimulation is not dependent on the PARylation of ALC1 itself (fig. S1C). Moreover, addition of purified PAR did not stimulate ALC1 (Fig. 1F), indicating that PAR binding alone is insufficient to stimulate ATPase activity. Thus, we propose that stimulation of ALC1 requires PARylated PARP1. PARP1 and NAD⁺ also stimulated the nucleosome-repositioning activity of ALC1 (Fig. 1G), thereby defining ALC1 as a PARP1-stimulated chromatin-remodeling enzyme.

To investigate the cellular functions of ALC1, we analyzed ALC1-associated immunocomplexes by means of mass spectrometry (MS). Chromatin extracts prepared from stable Flp-In-FLAG and Flp-In-ALC1 cell lines were subjected to immu-

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nonprecipitation with antibody to FLAG. Comparative MS analysis revealed the presence of proteins implicated in DNA repair but also in other cellular processes (Fig. 2A and fig. S2A) (23). Interactions with DNA-PKcs, Ku, and PARP1, as well as those with the DNA damage responsive XRCC1 and APLF proteins, were confirmed through immunoblot analyses (Fig. 2B). These interactions were largely abrogated by PARP inhibitor treatment, indicating that the association of ALC1 with these DNA repair proteins is dependent on PAR modifications.

The stimulation of ALC1 nucleosome-repositioning activity by PARP1 and the PAR-dependent interactions between ALC1 and several known DNA repair factors suggested a possible role for ALC1 in the DNA damage response. Indeed, we observed reversible mobilization of ALC1 from the soluble to chromatin-bound fraction in response to DNA damage induced by H_2O_2 treatment, which was largely dependent on active PAR synthesis (fig. S2B). Furthermore, we observed the colocalization of ALC1 with sites of active PAR synthesis in mouse 3T3 cells after

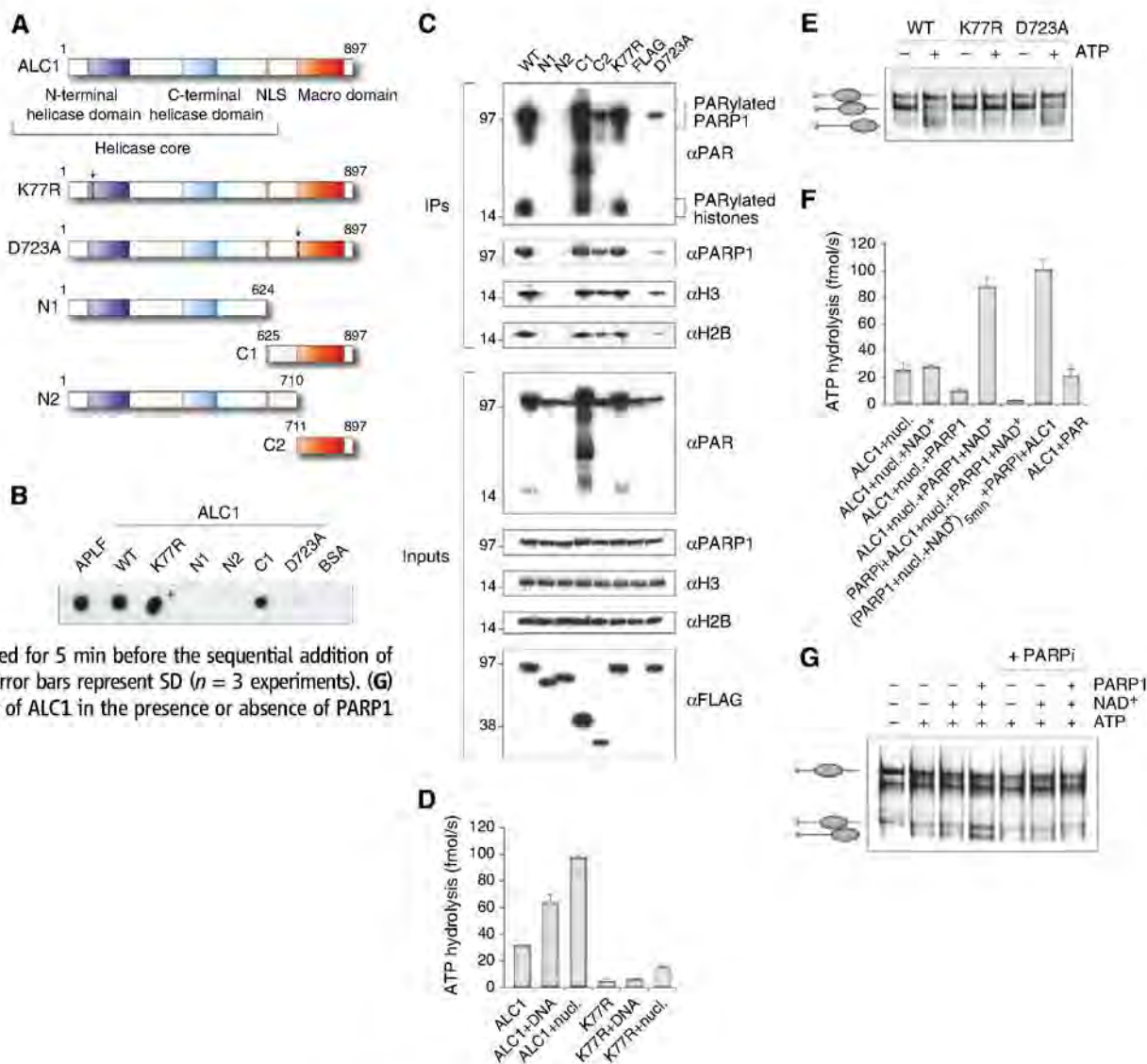
exposure to H_2O_2 (fig. S2C). To further investigate the role of ALC1 in the DNA damage response, we analyzed the recruitment of ALC1 to sites of DNA damage induced by laser micro-irradiation. Endogenous ALC1 efficiently localized to sites of laser-induced DNA breaks, as marked by γ H2AX staining (Fig. 3A). Recruitment of fluorescently tagged yellow fluorescent protein (YFP)-ALC1 to laser-induced DNA breaks was also observed in transiently transfected U2OS cells (Fig. 3B), which was abolished in cells treated with the PARP inhibitor (Fig. 3B and fig. S2E).

The kinetics of ALC1 recruitment to DNA damage sites closely mirrored the dynamics of PAR, which is known to be a short-lived modification. Live cell imaging revealed an almost instant mobilization of ALC1 to DNA damage sites, and a relatively transient retention of ALC1 at such regions (Fig. 3C and fig. S2F). Moreover, the ALC1 K77R mutant, which is defective for nucleosome sliding in vitro, exhibited prolonged retention at DNA damage sites as compared with WT ALC1 (Fig. 3, D and E). This was even more pronounced with the macro domain fragment of

ALC1 (C1 mutant), which persisted at DNA damage sites beyond 40 min (Fig. 3D). In contrast, the helicase core fragment (N2 mutant) was not mobilized to DNA damage sites, and the recruitment of the D723A mutant was severely impaired (Fig. 3D). Thus, ALC1 recruitment required macro domain-mediated recognition of PAR at sites of DNA damage. Conversely, the timely disengagement of ALC1 from DNA damage sites required the ATPase activity of its helicase core. Expression of the macro domain fragment (C1) also led to prolonged accumulation of the single-strand-break repair factor XRCC1 at sites of DNA damage (fig. S3A), potentially indicating delayed repair kinetics in the absence of ALC1-mediated chromatin remodeling.

We also assessed the sensitivity of ALC1-deficient cells to various DNA-damaging agents. U2OS cells that were stably reduced for ALC1 expression by an integrated ALC1 short hairpin RNA (shRNA) construct (Fig. 4A) were more sensitive toward H_2O_2 and the radiomimetic drug phleomycin but not to camptothecin (Fig. 4B). We conclude that ALC1 is a nucleosome-repositioning

Fig. 1. (A) Schematic representation of the WT and mutant ALC1 proteins. (B) Analysis of PAR binding in vitro. ALC1 proteins were dot-blotted onto a nitrocellulose membrane and incubated with 32 P-labeled PAR. (C) Immunoprecipitates from cells expressing WT or mutant FLAG-tagged ALC1 proteins blotted for PAR, PARP1, and core histones. (D) Quantification of ATPase activities. Error bars represent SD ($n = 3$ experiments). (E) Nucleosome-sliding activity of WT ALC1 and K77R and D723A mutants. (F) Quantification of ATPase activities. Where indicated, PARP1, NAD^+ , and nucleosomes were preincubated for 5 min before the sequential addition of PARP inhibitor and ALC1. Error bars represent SD ($n = 3$ experiments). (G) Nucleosome-sliding activity of ALC1 in the presence or absence of PARP1 and NAD^+ .



enzyme that is specifically targeted to sites of DNA damage through interaction with PAR and functions to regulate chromatin during DNA repair.

ALC1 was recently identified as a target oncogene within the 1q21 amplicon, which is the most frequent genetic alteration in human hepato-

cellular carcinoma (HCC) and occurs in 58 to 78% of primary HCC cases (24). Although the precise molecular impact of ALC1 overexpression is unclear, ALC1-overexpressing cells display increased colony formation in soft agar and tumorigenicity in nude mice (25). To assess the possible

consequences of ALC1 overexpression, we analyzed induction of H2AX phosphorylation (a DNA damage marker) in ALC1-overexpressing and control cells after phleomycin treatment (fig. S4A). No measurable differences in γ H2AX profiles were observed between untreated control and ALC1-overexpressing cells. In contrast, phleomycin induced H2AX phosphorylation in 44 to 50% of control cells, whereas 80 to 93% of ALC1-overexpressing cells exhibited H2AX phosphorylation (Fig. 4C and fig. S4B). Phleomycin treatment also consistently produced longer tails (more damage) in alkaline Comet assay in ALC1-overexpressing cells as compared with control cells (Fig. 4, D and E). This phenotype was abrogated by the K77R ALC1 mutation (Fig. 4C) and was unaffected by PARP inhibitor treatment (fig. S4C), suggesting that the phenotype required ALC1 chromatin-remodeling activity but not its recruitment to the sites of DNA damage. Furthermore, the phenotype was specific to phleomycin and could not be observed with ionizing radiation or H_2O_2 (fig. S4D and E), possibly reflecting a difference in the accessibility of chromatin-embedded DNA to these DNA-damaging agents.

Fig. 2. (A) Identification of ALC1-interacting partners by means of MS. **(B)** Western blotting of ALC1 immunoprecipitates from transiently transfected 293T cells for known DNA repair factors.

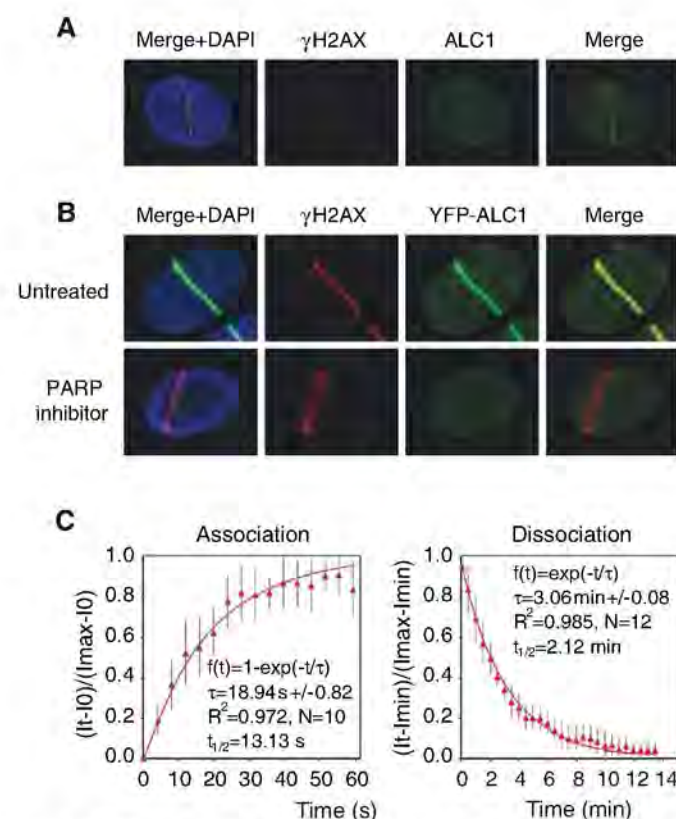
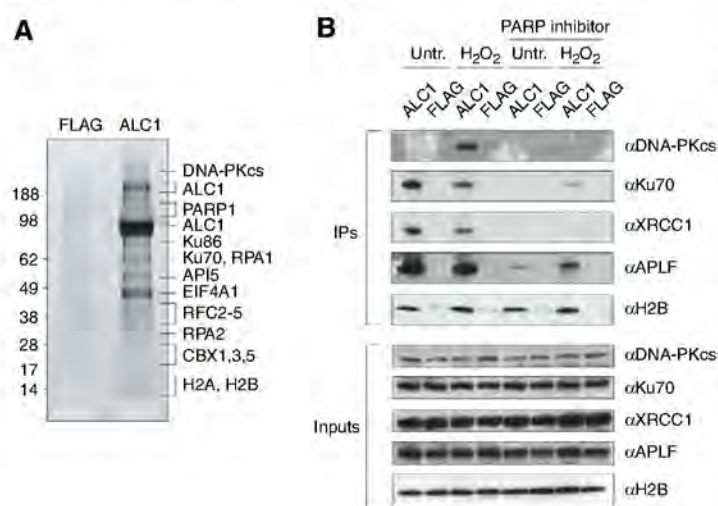
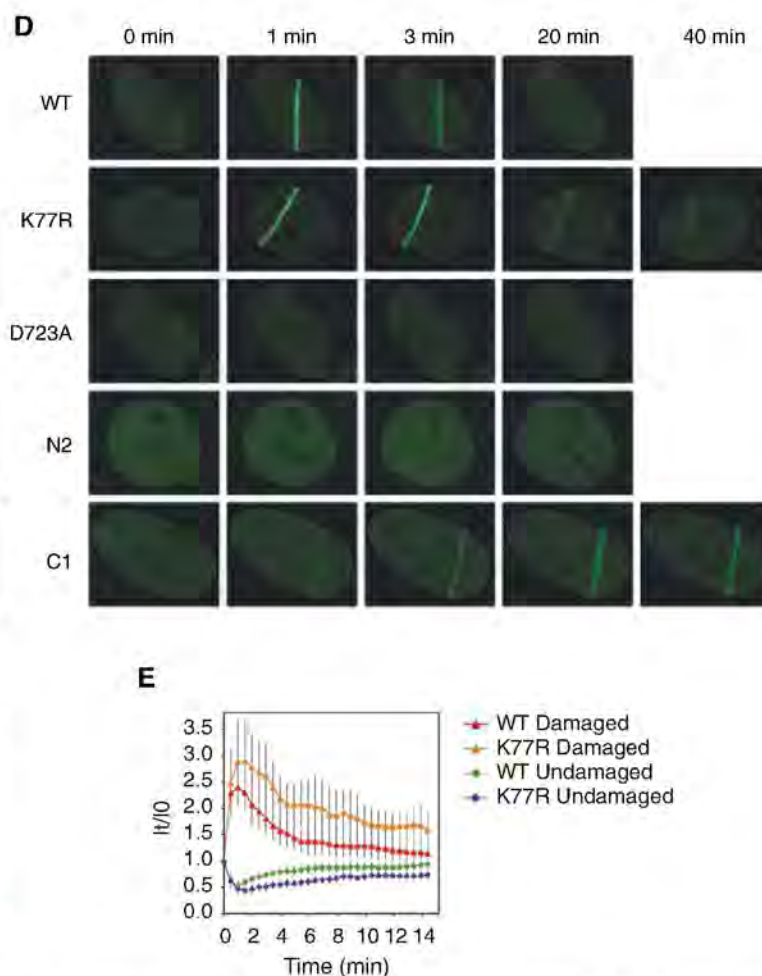
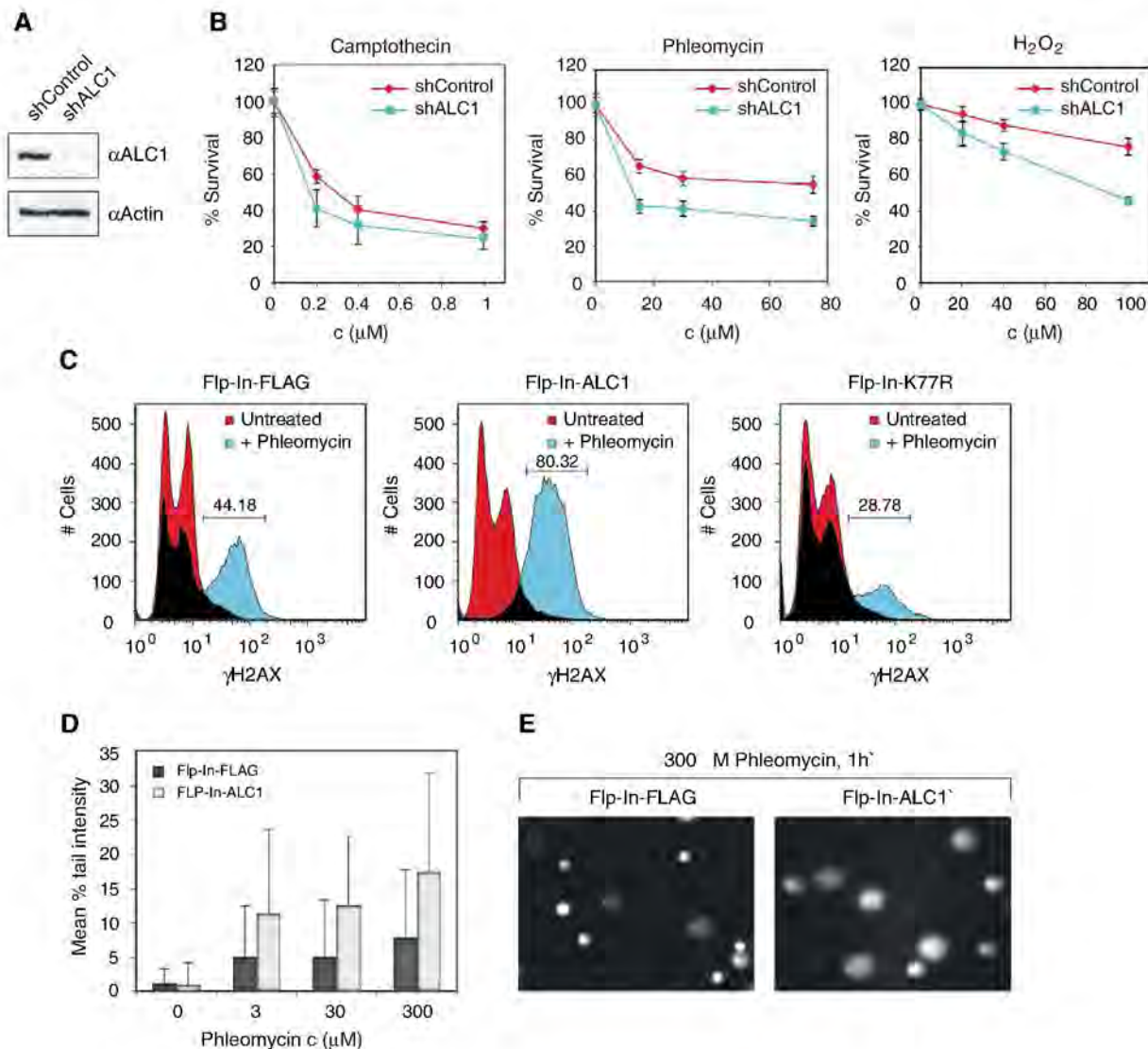


Fig. 3. (A) Recruitment of endogenous ALC1 to sites of laser-induced DNA damage, 1 min after laser damage. **(B)** Recruitment of the transiently expressed YFP-ALC1 to laser-induced DNA breaks, with and without PARP inhibitor. **(C)** Kinetics of ALC1 association and dissociation from DNA breaks.



(D) Recruitment of the ALC1 derivatives and mutants to laser-induced DNA breaks. **(E)** Comparison of the WT and K77R mutant ALC1 kinetics at laser-induced DNA breaks.

Fig. 4. (A) Knockdown efficiency of ALC1 in U2OS-stable shRNA cell lines. **(B)** Sensitivity of ALC1-deficient cells to DNA-damaging agents. Data are averaged values from 3 experiments. **(C)** γ H2AX levels assessed by fluorescence-activated cell sorting analysis in cell lines of the indicated genotype, 1 hour after 300 μ M phleomycin treatment. **(D)** DNA damage levels in cells ($n > 200$ cells) of the indicated genotype assessed by means of Comet assay. Error bars represent SD. **(E)** Representative images of control and ALC1-overexpressing cells analyzed by means of Comet assay.



Indeed, these results are in agreement with the observation that the structurally related DNA-damaging agent bleomycin induces DNA breaks in the linker region but not in core DNA and therefore acts preferentially on relaxed chromatin (25). We thus conclude that ALC1 overexpression leads to chromatin relaxation and an increased susceptibility to phleomycin-induced DNA damage. These findings provide a key insight into the molecular consequence of ALC1 deregulation, emphasizing the importance of chromatin reorganization as a critical element in genome stability and cancer.

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- Research in the labs of S.J.B., S.P.J., S.C.W., I.A., and M.S. is supported by Cancer Research UK. The lab of T.O.-H. is funded by a Wellcome Trust Senior Fellowship 064414. S.P.J. is also funded by the UK Biotechnology and Biological Sciences Research Council, Medical Research Council (UK), the Wellcome Trust, and the European Union (EU Projects GENICA and DNA Repair). D.A. is supported by the European Molecular Biology Organization. S.E.P. is supported by the Human Frontier Science Program Organization. S.C.W. and I.A. are also funded by the Louis-Jeantet Foundation. The authors declare no conflict of interest.

Supporting Online Material

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Materials and Methods
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References

5 June 2009; accepted 17 July 2009
Published online 30 July 2009;
10.1126/science.1177321
include this information when citing this paper.

Fundamental Evolutionary Limits in Ecological Traits Drive *Drosophila* Species Distributions

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Species that are habitat specialists make up much of biodiversity, but the evolutionary factors that limit their distributions have rarely been considered. We show that in *Drosophila*, narrow and wide ranges of desiccation and cold resistance are closely associated with the distributions of specialist and generalist species, respectively. Furthermore, our data show that narrowly distributed tropical species consistently have low means and low genetic variation for these traits as compared with those of widely distributed species after phylogenetic correction. These results are unrelated to levels of neutral variation. Thus, specialist species may simply lack genetic variation in key traits, limiting their ability to adapt to conditions beyond their current range. We predict that such species are likely to be constrained in their evolutionary responses to future climate changes.

Specialist species within particular climatic zones and/or dependent on particular hosts make up much of biodiversity (1), but generally investigations have not considered whether evolutionary factors may limit the distributions of these species (2, 3). Models predicting climate change suggest that it is crucial to determine how species will evolve and contract or expand from their current distributions, particularly in the case of threatened climate specialists. Distributional patterns may be explained by the fact that species simply lack the appropriate genetic variation in key traits necessary to adapt to conditions beyond their distributions and thus are constrained by a fundamental genetic limit (4). Such a lack of genetic variation contradicts the theory that traits are highly variable (5), which originated from quantitative genetic theory that shows mutational variance to be large enough to maintain genetic variation within traits (6).

In particular, the idea that genetic variance might limit evolutionary responses was supported by the fact that certain plant species exhibited phenotypic variation for resistance to soil contamination and could successfully colonize mine tailings contaminated with heavy metals (7). Species or populations unable to evolve higher levels of metal resistance were limited by low levels of genetic variation for this trait. Few studies had gone on to further test this prediction until it was suggested that a lack of appropriate variation for desiccation resistance might be linked to the distribution of two rain forest *Drosophila* species (3, 8). These desiccation-sensitive species did not respond to persistent selection for increased desiccation resistance, whereas cross-generation comparisons suggested that this was due

to very low genetic variation for this trait (8, 9) in contrast to other widespread *Drosophila* species (10).

Environmental variables are tightly associated with the distribution of *Drosophila* and insects more generally (11). It is well documented that species distributions become narrower toward the tropics (Rapaport's rule) (12). One hypothesis to explain this pattern and which has empirical support is the climatic variability hypothesis (12), which proposes that species with greater physiological tolerance to climatic variables will be able to extend their distribution to higher latitudes. This leaves species that are unable to increase their tolerance restricted to the tropics.

We extend the climatic variability hypothesis and suggest that levels of genetic variation in key

ecological traits are driving differences in physiological tolerances between tropical and widespread *Drosophila* species. To examine this, we initially compared patterns of mean resistance to two stresses, desiccation and cold, for 30 species (13). Both resistance traits have been closely linked to differences in species distributions [cold resistance in (14–16) and desiccation resistance in (15, 17)]. We found that species restricted to the tropics showed low levels of resistance to both desiccation and cold in comparison with more widespread temperate species (Fig. 1). Consequently, there was a strong association between levels of resistance and tropical versus widespread distribution, with the exception of *Drosophila funebris*, which is adapted to cold environments (Fig. 1). These data are in accord with the climatic variability hypothesis and with studies that also show that levels of cold resistance in species match expectations on the basis of climatic data (18, 19). Furthermore, these results confirm that cold and desiccation are important variables influencing distributional patterns in *Drosophila* species. Non-drosophilid tropical insects also have a much narrower range of thermal optima (12).

To establish whether low genetic variation in resistance traits influences tropical and widespread distributions, we estimated genetic variation (mostly as h_n^2 , the narrow sense heritability) in five tropically restricted and five widespread *Drosophila* species. For most traits, pedigree information was analyzed with statistical animal models (13, 20) to generate small SEs around heritability estimates. Patterns of genetic variation in the traits of cold and desiccation resistance were consistent with our hypothesis; low levels of genetic variation

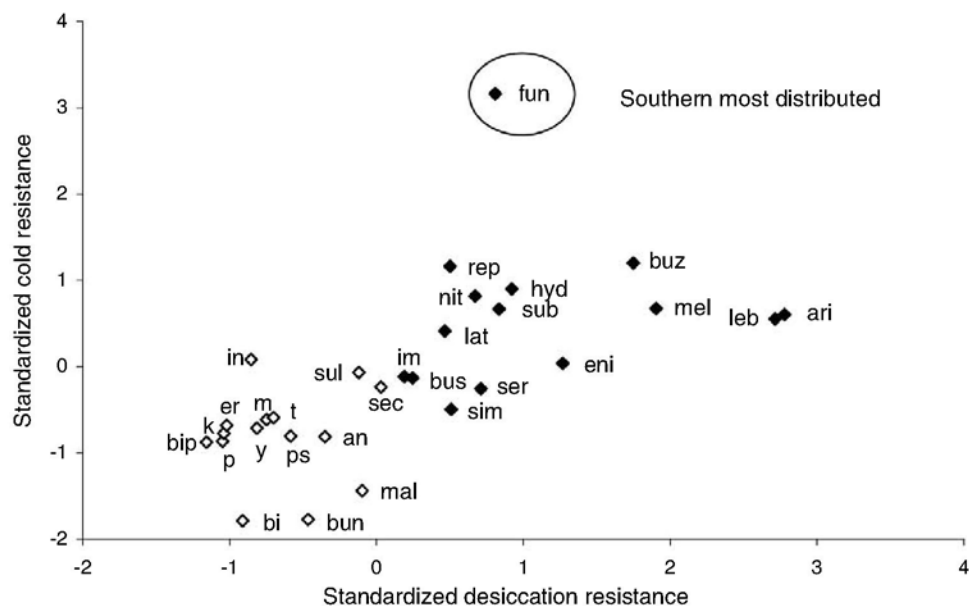


Fig. 1. Standardized cold resistance plotted against standardized desiccation resistance for *Drosophila* species. ♦ indicates species with a widespread distribution; ◇ indicates species with a restricted/tropical distribution. an, *ananassae*; ari, *arizonensis*; bip, *bipunctata*; bi, *birchii*; bus, *busckii*; bun, *bunnanda*; buz, *buzzatii*; er, *erecta*; eni, *enigma*; fun, *funebris* (cold adapted); hyd, *hydei*; im, *immigrans*; in, *inornata*; k, *kikkawai*; lat, *lativittata*; leb, *lebanonensis*; mal, *malerkotliana*; m, *mauritiana*; mel, *melanogaster*; nit, *nitidithorax*; p, *pauistorum*; ps, *pseudoananassae*; rep, *repleta*; sec, *sechellia*; ser, *serrata*; sim, *simulans*; sub, *subobscura*; t, *teisseri*; y, *yakuba*; and sul, *sulfurigaster*. The outlier noted in the text is circled.

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were found in all tropical species, as indicated by low heritability estimates (Fig. 2). Those cases in which we observed only small and nonsignificant estimates of h_n^2 were unlikely to reflect a lack of power because we obtained low SEs for most comparisons. In contrast, we observed significant levels of variation in all widespread species, and the difference in h_n^2 between tropical and widespread species overall was greater than twofold for both desiccation and cold resistance (table S1). Patterns of additive genetic variance (V_A) also matched that of h_n^2 , highlighting that specialization to tropical environments may reflect low genetic variation in key ecological traits. Fundamental limits in genetic variation therefore appear to be driving distribution limits in these *Drosophila* species.

The most commonly invoked explanation for such observed low levels of genetic variation in traits is that they are subject to directional selection that causes the fixation of favored alleles (21). However, if this is true then low genetic variation should be coupled with high (directionally selected) trait means if high resistance levels are favored. Instead, if genetic variation is directly involved in limiting species distributions, we would expect low genetic variation to be coupled with low trait means, whereas species with high levels of genetic variation should not be constrained in this way (22). Under these predictions, the mean expression of a distribution-limiting trait and levels of genetic variation for that trait should be correlated, as was observed in this study (Fig.

2). If experimental error was swamping estimates of h_n^2 , particularly in the restricted species, an association between environmental variance and trait mean would be expected; this was not the case for either desiccation ($r^2 = 0.05$, $P = 0.51$) or cold ($r^2 = 0.26$, $P = 0.14$) resistance.

To ensure that phylogenetic history was not responsible for the observed patterns in trait mean, h_n^2 , and species distribution, we generated phylogenetic independent contrasts (PICs) (13, 23). PICs generate statistical independence within a data set by standardizing trait values by the branch length in two sister species. Each comparison results in an independent contrast, and consequently the number of contrasts equals $n - 1$. We used a highly resolved phylogenetic tree (fig. S1), created on the basis of two mitochondrial (*COII* and *ND5*) and two nuclear (*ADH* and *hb*) genes, to phylogenetically correct our estimates of h_n^2 , V_A , and trait mean. The strength of the relationships decreased slightly after correction for phylogeny but were still significant (Table 1). When PICs for cold and desiccation resistance were plotted, a strong positive and linear association remained. Thus, trait mean was closely associated with levels of genetic variation (Fig. 2 and table S1), emphasizing that levels of genetic variation for cold and desiccation resistance, rather than phylogeny, are driving species distributions.

Population processes such as inbreeding or drift could alter levels of genetic variation and explain the observed patterns. However, if this was true we would expect low levels of genetic variation in all

traits. To test this, we estimated h_n^2 for wing size (13). In contrast to the stress resistance traits, we saw no consistent association between h_n^2 or V_A and trait mean between restricted and widespread species. All estimates of both h_n^2 and V_A differed significantly from zero in all species (table S1). We observed no relationship between mean wing size and h_n^2 ($r^2 = 0.001$, $P = 0.92$). We also tested for a relationship between wing size and mean resistance because body size could influence trait resistance (24); however, we did not detect a relationship between any of the traits (for desiccation, $r^2 = 0.29$, $P = 0.10$; for cold, $r^2 = 0.10$, $P = 0.36$). Furthermore, in some species microsatellite repeat variation has been characterized. For these species, it was high and comparable for species that are widespread and those that are climatically specialized (25).

It is unclear what mechanisms produce these patterns. Hypotheses involving asymmetrical gene flow that might account for evolutionary stasis do not predict that low levels of genetic variation will limit evolutionary responses (22). The rate of mutation seems unlikely to limit variation in our tropical species because we would expect equivalent levels of variation to arise through mutation if desiccation resistance is influenced by similar genes across species. Our results may be caused by a loss of genetic variance through DNA decay (22); if selection on these traits is weak or essentially neutral, theory suggests that mutations will accumulate that could potentially result in loss of function of genes, and hence traits (26). The hypothesis that the loss of underlying

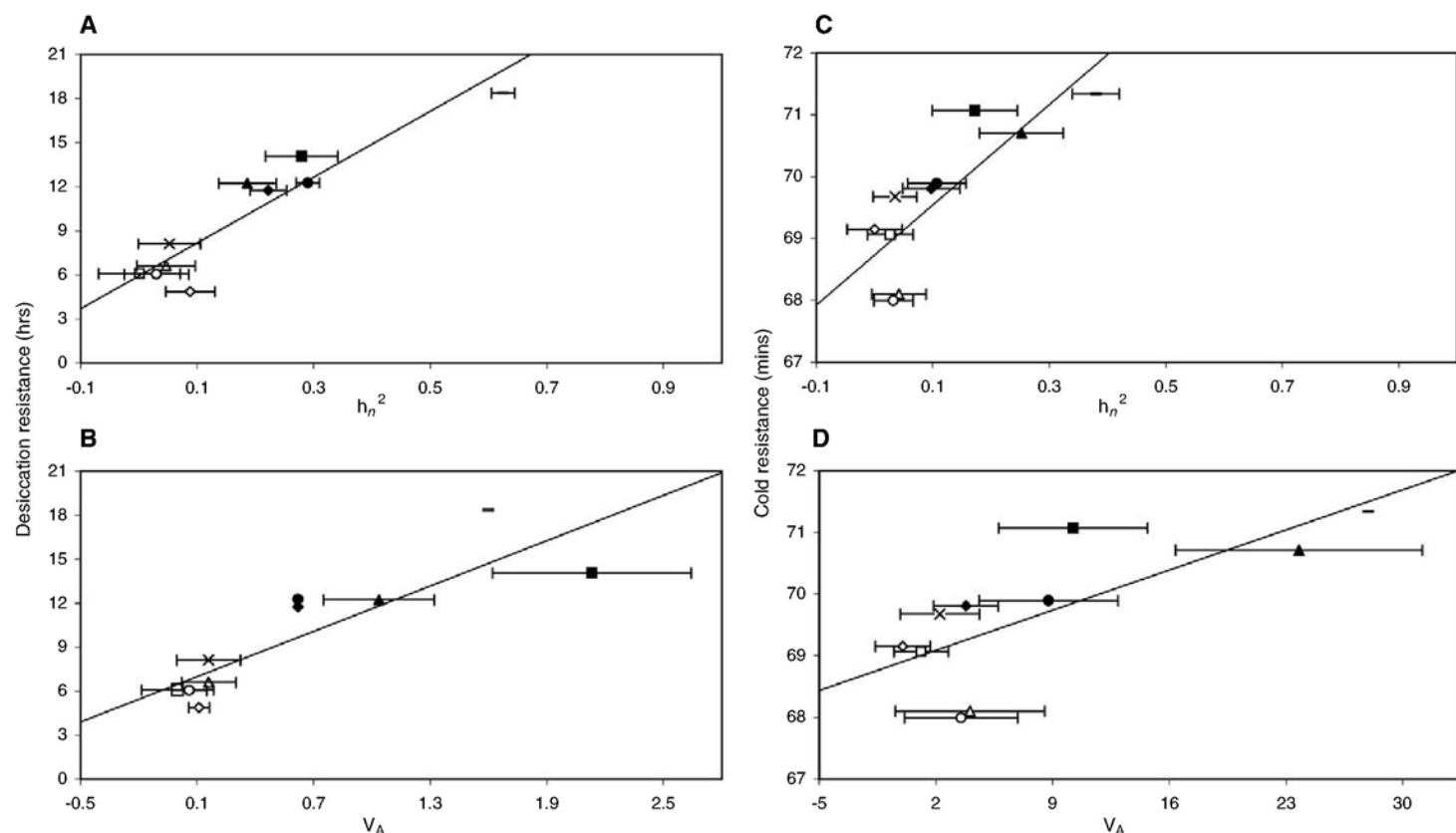


Fig. 2. Species comparison with trait mean and genetic variance estimates. —, *D. melanogaster*; ●, *D. simulans*; ▲, *D. repleta*; ■, *D. hydei*; ◆, *D. serrata*; ○, *D. birchii*; △, *D. bunnanda*; □, *D. pseudoananassae*; ×, *D. sulfurigaster*;

and ◇, *D. bipectinata*. (A) h_n^2 versus desiccation resistance. (B) V_A versus desiccation resistance. (C) h_n^2 versus cold resistance. (D) V_A versus cold resistance. Error bars reflect one SE.

Table 1. Regression analyses for trait means onto genetic variance estimates for desiccation and cold resistance. Regressions were computed on both the original unstandardized data set and the PICs.

h_n^2	Desiccation		Cold	
	Original	PIC	Original	PIC
Slope $\pm$ SE	21.69 $\pm$ 0.92	17.97 $\pm$ 1.23	7.76 $\pm$ 0.60	6.13 $\pm$ 0.54
r^2	0.87	0.75	0.67	0.64
P	<0.001	0.03	0.01	0.01
V_A				
Slope $\pm$ SE	5.21 $\pm$ 0.33	4.20 $\pm$ 0.43	0.09 $\pm$ 0.01	0.07 $\pm$ 0.01
r^2	0.76	0.56	0.55	0.48
P	<0.001	0.04	0.01	0.03

genetic variability through DNA decay leads to ecological specialization has been proposed to occur in some bacterial systems (27). The tropical species may be evolutionarily derived, or else the more widespread species are derived after activation of resistance, perhaps after gene duplication or the development of novel resistance pathways. Trade-offs might also be involved if they produce strong directional selection for sensitivity to cold and desiccation in tropically restricted species.

Our analysis supports the hypothesis that specialization to tropical environments in *Drosophila* species reflects fundamental genetic limits in ecologically important traits. We show that genetic variation in tropical *Drosophila* species for both desiccation and cold resistance is consistently low, which is in contrast to widely distributed species. Whether other tropically restricted species share a similar evolutionary fate remains to be determined. However, evidence suggests that an absence of genetic variation may be limiting distributions in other systems (2). Further work examining the genetic architecture of traits that may be linked to species distributions will be important for the assessment of the future evolutionary potential of species. If species distributions are commonly driven by genetic limits, the consequences of climate change on biodiversity may be greater than previously assumed. It is worth considering heat resistance as well as cold and desiccation resistance within this context, particularly because recent research has highlighted the pending threat of increases in temperature on the persistence of tropical species (12, 28, 29).

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- We thank the Australian Research Council and the Commonwealth Environmental Research Fund for financial support. We also thank M. Blacket for molecular work; A. Miller for assistance with the phylogenetic analysis; K. Mitchell, C. Doig, T. Kristensen, J. Overgaard, L. Carrington, K. Lein, and X. Du for technical support; and reviewers for constructive comments.

Supporting Online Material

www.sciencemag.org/content/full/325/5945/1244/DC1
Materials and Methods

Fig. S1

Tables S1 and S2

References

27 April 2009; accepted 17 July 2009
10.1126/science.1175443

Common Regulatory Variation Impacts Gene Expression in a Cell Type–Dependent Manner

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Studies correlating genetic variation to gene expression facilitate the interpretation of common human phenotypes and disease. As functional variants may be operating in a tissue-dependent manner, we performed gene expression profiling and association with genetic variants (single-nucleotide polymorphisms) on three cell types of 75 individuals. We detected cell type–specific genetic effects, with 69 to 80% of regulatory variants operating in a cell type–specific manner, and identified multiple expressive quantitative trait loci (eQTLs) per gene, unique or shared among cell types and positively correlated with the number of transcripts per gene. Cell type–specific eQTLs were found at larger distances from genes and at lower effect size, similar to known enhancers. These data suggest that the complete regulatory variant repertoire can only be uncovered in the context of cell-type specificity.

Variation influencing gene expression can manifest itself as gene expression differences among populations, among individuals in a population, among tissues, and in response to environmental factors. The genetic basis of the first two types of gene expression variation has been investigated, with the quantification of mRNA in one tissue and the identification of genetic variants correlated with the variation of expression quantitative trait loci (eQTLs) in a single or multiple populations (1–7). The complexity in higher eukaryotes, however, results in a vast set of highly

specialized cell types and tissues. Some genes exhibit ubiquitous patterns of expression; others display tissue-specific activity (8–10). Although some studies have identified eQTLs in human (11–13) and mammalian tissues (14, 15), we know of no systematic study that has compared eQTLs across different cell types while controlling for confounding associations (population samples, differences in technology, or statistical methodology). Documenting tissue-specific genetic control of gene expression variation may connect cellular activities in health and disease (11, 13, 16). Efforts to use

genomic information to interpret the biological effects of such variants are hindered by the limited availability of the relevant human tissues.

We investigated 85 individuals of the GenCord project (a collection of cell lines from umbilical cords of individuals of Western European origin) to identify *cis* eQTLs involved in gene expression variation in three cell types: primary fibroblasts, Epstein-Barr virus-immortalized B cells (lymphoblastoid cell lines or LCLs), and T cells. Umbilical cord was chosen because it is readily available and allows the acquisition of multiple cell types. Sample collection was performed on full-term or near-full-term pregnancies to ensure the homogeneity of sample ages.

mRNA levels were quantified in primary fibroblasts, LCLs, and primary T cells for 48,804 probes with the Illumina WG-6 v3 expression array. We analyzed data from 22,651 probes uniquely mapping to 17,945 autosomal RefSeq genes [15,596 Ensembl genes from the National Center for Biotechnology Information (NCBI) Reference Sequence]. Samples were genotyped on the Illumina 550K single-nucleotide polymorphism (SNP) array. After quality control was performed (SNPs with missing data were removed) and a minor allele-frequency filter ($MAF \geq 5\%$) was applied, 394,651 SNPs were used for association testing. Principal components analysis (PCA) detected 10 potential outlier individuals from the genotype data (17) (fig. S1), which subsequently were removed from the analysis. eQTL discovery and all other properties of the results for 75 versus 85 individuals were almost identical (fig. S2).

We explored associations in *cis*, by testing all SNPs within a 2-megabase window centered on the transcription start site (TSS) of a gene. Using the Spearman rank correlation (SRC), we tested for associations between SNP genotype and mRNA levels (intensities), after normalization and transformation. SRC performs similarly to linear regression (7), but is not sensitive to outliers in the gene expression data, which reduce power. A total of 6,083,130 tests were performed, and significance thresholds for each gene were assigned through 10,000 permutations of expression values, as described (6, 7, 18). For 75 individuals at the 0.001 permutation threshold (PT), we discovered 427, 442, and 430 genes with significant *cis* associations in fibroblasts, LCLs, and T cells, respectively, with an estimated false-discovery rate (FDR) of 4% (fig. S2, A and B; and tables S1 and S2). For the less stringent PT of 0.01, we discovered 2146, 2155,

and 2046 genes with an estimated FDR of 7% (Fig. 1) (19). The range of allelic effects was estimated by comparing the median expression in the two homozygote classes for the eQTL SNPs, and the 95% confidence limits of fold change were between 1.07 and 2.65 (fig. S7).

We assessed whether we can replicate the LCL eQTLs from previous studies. eQTLs from the CEU HapMap LCLs [selected by the CEPH (Centre d'Etude du Polymorphisme Humain) and from Utah (CEU)] (7, 17) were replicated in the GenCord LCLs. Both populations are of European descent and share similar allele frequency spectra. Because of the differences in probe sequence content between the Illumina v1 array (used for the CEU HapMap) and the v3 array (used here), we could only compare a small subset of those SNP-probe associations. Of the 5898 SNP-probe pairs surviving the 0.001 PT in the CEU HapMap sample, 137 SNP-probe pairs (44 probes, some associated with multiple SNPs) were also tested in GenCord LCLs. Of the 137 SNP-probe tests, 114 had P values of less than 0.001 (83%) (fig. S3). Therefore, previously detected eQTLs were well replicated, despite the long separation time between tests of these cell lines, which demonstrated the stability of transformed B cells.

We interrogated the cell-type specificity of regulatory effects by exploring genes with *cis* eQTLs that were (i) shared in all three cell types, (ii) shared in two cell types, and (iii) cell-type specific. At the 0.001 PT, we identified a nonredundant set of 1007 genes with *cis* eQTLs of which 86 (8.5%) were shared among all three cell types, 120 (12.0%) were shared in two of the cell types, and 801 (79.5%) were cell-type specific (tables

S1 and S2). The proportion of cell type-specific eQTLs was similar to previous estimates of eQTL tissue specificity and alternative splicing reported in a study interrogating two tissue types sampled, however, from different individuals (20).

The degree of eQTL sharing across cell types (fig. S4) is overestimated because the eQTLs for the same gene are not necessarily identical genetic variants (see below). Of the genes with *cis* eQTLs common to two or more cell types, 124 (12.3%) were shared between fibroblasts and LCLs, 121 (12.0%) were shared between fibroblasts and T cells, and 133 (13.2%) were shared between LCLs and T cells. Increased eQTL sharing between LCLs and T cells is most likely due to the similarity of these cell types. We observed a striking prominence of tissue specificity with 268 (26.6% of total), 271 (26.9%), and 262 (26.0%) of gene eQTL associations found only in fibroblasts, LCLs, and T cells, respectively.

To test if the eQTL cell-type specificity arises from differential expression between cell types, we compared medians and variance of gene expression. We found that genes with cell type-specific eQTLs had significantly higher expression variance in the eQTL cell type (Mann-Whitney U test, $P < 0.0001$ for all comparisons). Medians for the same genes were either marginally significantly different or not different, which means that genes included in this analysis were largely expressed in all cell types. This suggests that the majority of cell-type specificity is not a result of differential gene expression levels between cell types, but due to cell type-specific use of regulatory elements.

To dissect the overlap of *cis* eQTLs across cell types, we compared the direction of the allelic effect for eQTLs significant in two or more cell types. The

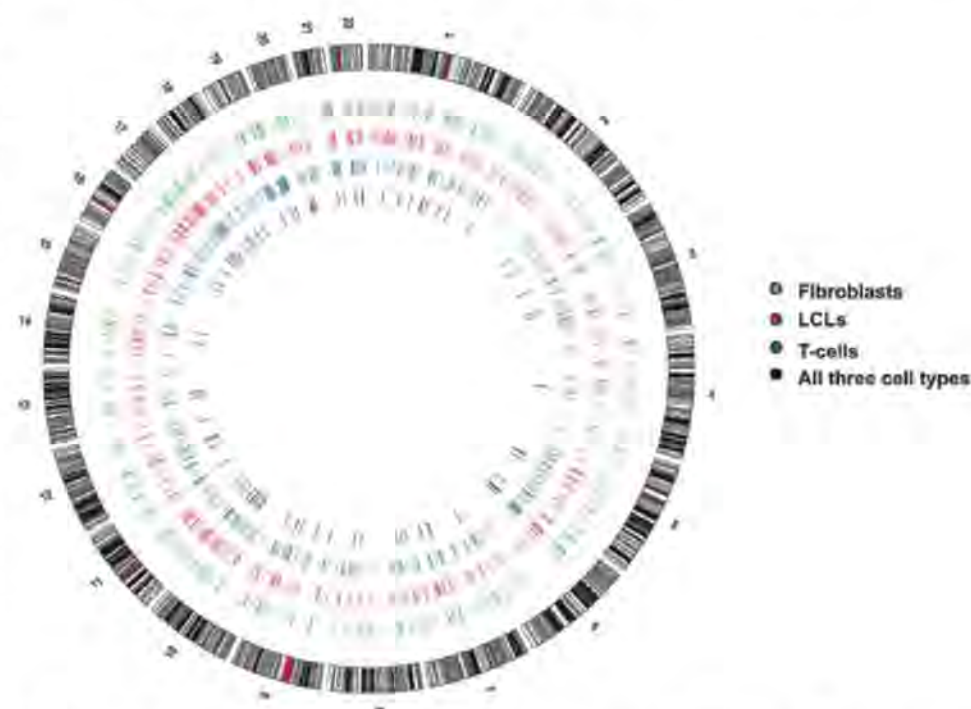


Fig. 1. Genome-wide map of *cis* eQTLs in three cell types; *cis* eQTLs at 0.001 PT are shown as color-coded lines on their corresponding chromosomal location. Internal black lines represent genes with eQTLs in all cell types.

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direction (sign of Spearman ρ) was in complete agreement for all pairwise cell-type comparisons at the 0.001 PT (fig. S5). Thus, regulatory variants are active across cell types in the same manner. To assess the strength of cell-type specificity, we performed repeated-measures ANOVA (RMA). Cell-type specificity was reflected in the SNP $\times$ cell type–interaction term (the part of the equation that tests SNP's effects dependent on the cell type), where cell type–specific associations are expected to be significant. We found 61% enrichment of low P values in cell type–specific eQTLs [quantified by estimation of FDR (21)] (fig. S6). No enrichment was observed for cell type–shared eQTLs. RMA, however, is limited, as the power to detect an interaction term is never maximized because of the lack of allelic effect reversal between cell types.

We further used allele specific–expression assays to validate a subset of cell type–specific eQTLs. We measured the ratio of the two alleles of transcript SNP in RNA samples of individuals who were double heterozygotes for both the eQTL and the transcript SNP. For 35 transcript SNPs (7 from fibroblasts, 14 from LCLs, and 14 from T cells), we observed extensive allelic imbalance (ratio of the abundance of the transcripts of the two alleles) for the eQTL cell type; this was highly significantly different from the ratios of the same SNPs in cell types without the eQTL (paired t test, $P = 5.6 \times 10^{-7}$) (fig. S7). Therefore, the eQTL cell-type specificity has been experimentally confirmed.

Shared associations among individual cell types increased slightly at relaxed significance thresholds for one cell type, because of the so-called “winner’s curse” (22–24) (fig. S8). This states that the effect sizes discovered when applying specific statistical significance thresholds are inflated compared with the true effect size. Consequently, the discovery sample usually achieves higher significance than replication samples. Even with relaxed thresholds, however, over half of the associations we detected remain cell-type specific. We selected significant SNP–probe pairs from one cell type and explored their nominal (uncorrected) P -value distribution in the other two cell types. These distributions were enriched for low P values and so reflected those associations that are shared between cell types (Fig. 2). When we removed SNP–probe associations with significant associations in the secondary cell type (i.e., shared associations at the same and lower significance threshold), the resulting nominal P -value distributions demonstrated only small enrichment for low P values. We quantified the fraction of significant cis eQTLs from one cell type that is not nominally significant ($P > 0.05$ before correction) in either of the other two cell types. We estimate that 54, 50, and 54% of cis eQTLs in fibroblasts, LCLs, and T cells, respectively, are cell-type specific, which amounts to 69% of all cis eQTLs at 0.001 PT. Therefore, the limited overlap of cis eQTLs between cell types is unlikely to result from the winner’s curse and supports the conclusion that a substantial fraction of eQTLs are cell-type specific.

As previously observed (7, 25), we found that the strength and density of cis eQTLs decay sym-

metrically with increasing distance from the corresponding gene’s TSS (Fig. 3A). To better understand the independent regulatory effects, we mapped eQTLs into recombination hotspot intervals and, subsequently, further controlled for linkage disequilibrium (19, 26). At the 0.001 PT, we observed that

5.1% of associated genes have more than one independent interval carrying an eQTL (table S3). To further dissect the regulatory variant sharing between genes, we compared the overlap of independent eQTLs (i.e., regulatory intervals, rather than genes) across cell types. When all intervals

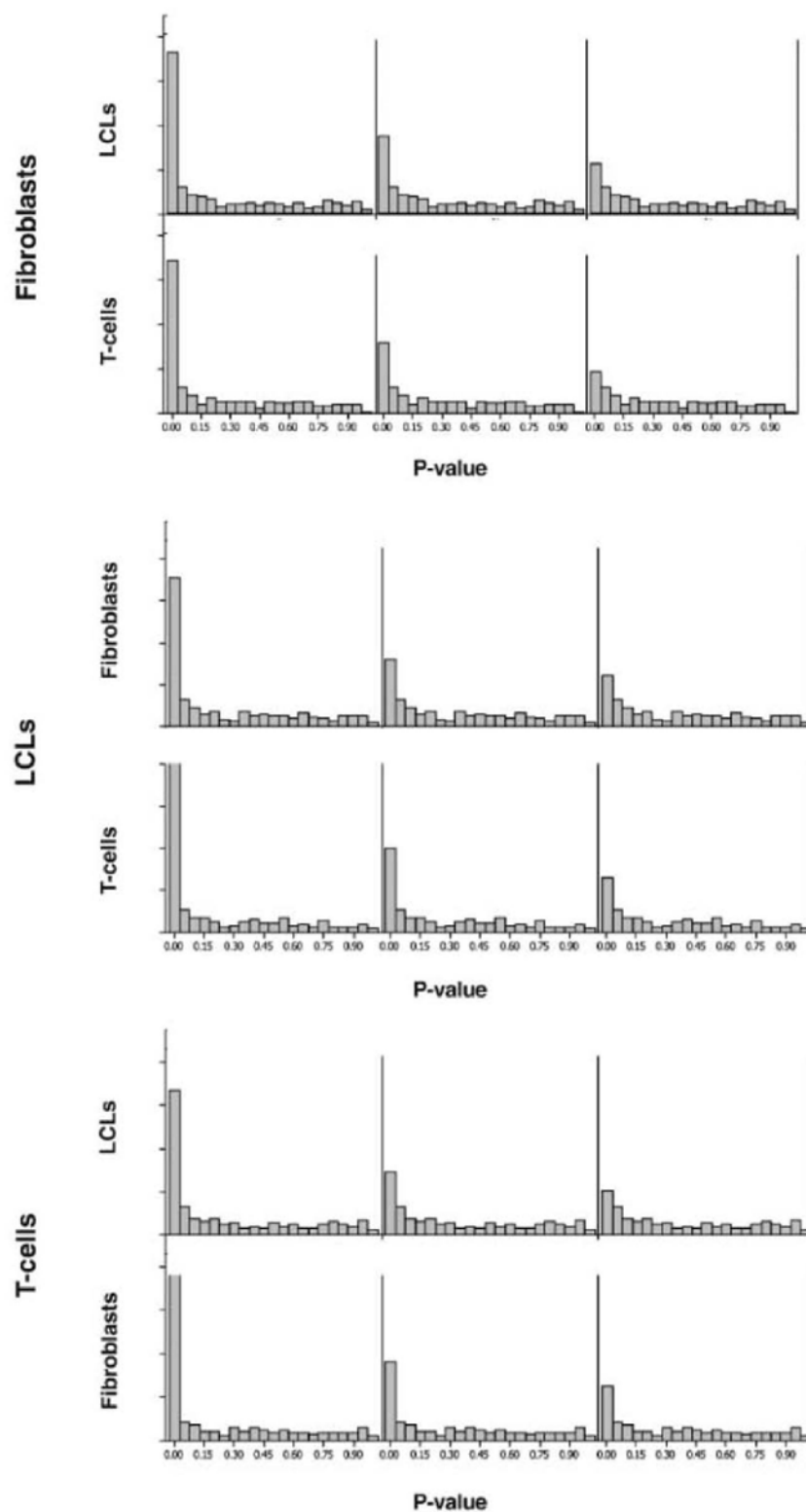


Fig. 2. SNP-probe pair nominal (uncorrected) P -value distributions for the two secondary cell types (label next to y axis) conditional on the reference cell type eQTL (leftmost label), significant at 0.001 PT are shown. The panels on the horizontal axis correspond (from left to right) to (i) the full P -value distribution of the secondary cell type, (ii) the P -value distribution after excluding significant eQTLs at 0.001 PT in secondary cell type, and (iii) similar to (ii) at 0.01 PT.

with a 0.001 PT eQTL were considered, only 6.9% were found to be shared across all three cell types. In addition, 9.7% were shared in two cell types, and 83.4% were cell-type specific (Fig. 4A and table S4). The degree of overlap increased as independent eQTLs for genes with shared expression associations across cell types were analyzed

(Fig. 4, B and C). In all cases, however, a substantial fraction of independent eQTLs were cell-type specific.

Cell type-shared eQTLs tend to have larger effects (Spearman ρ) and higher significance and to cluster tightly around the TSS (Fig. 3B and fig. S9). Cell type-specific eQTLs, however, have

lower effect sizes and are more widely distributed around the TSS (Fig. 3C). This is in agreement with the finding that enhancer elements, which tend to be found at greater distances from the gene, show greater tissue-specificity than basic regulatory elements (27). The number of eQTLs per gene was significantly correlated with number of tran-

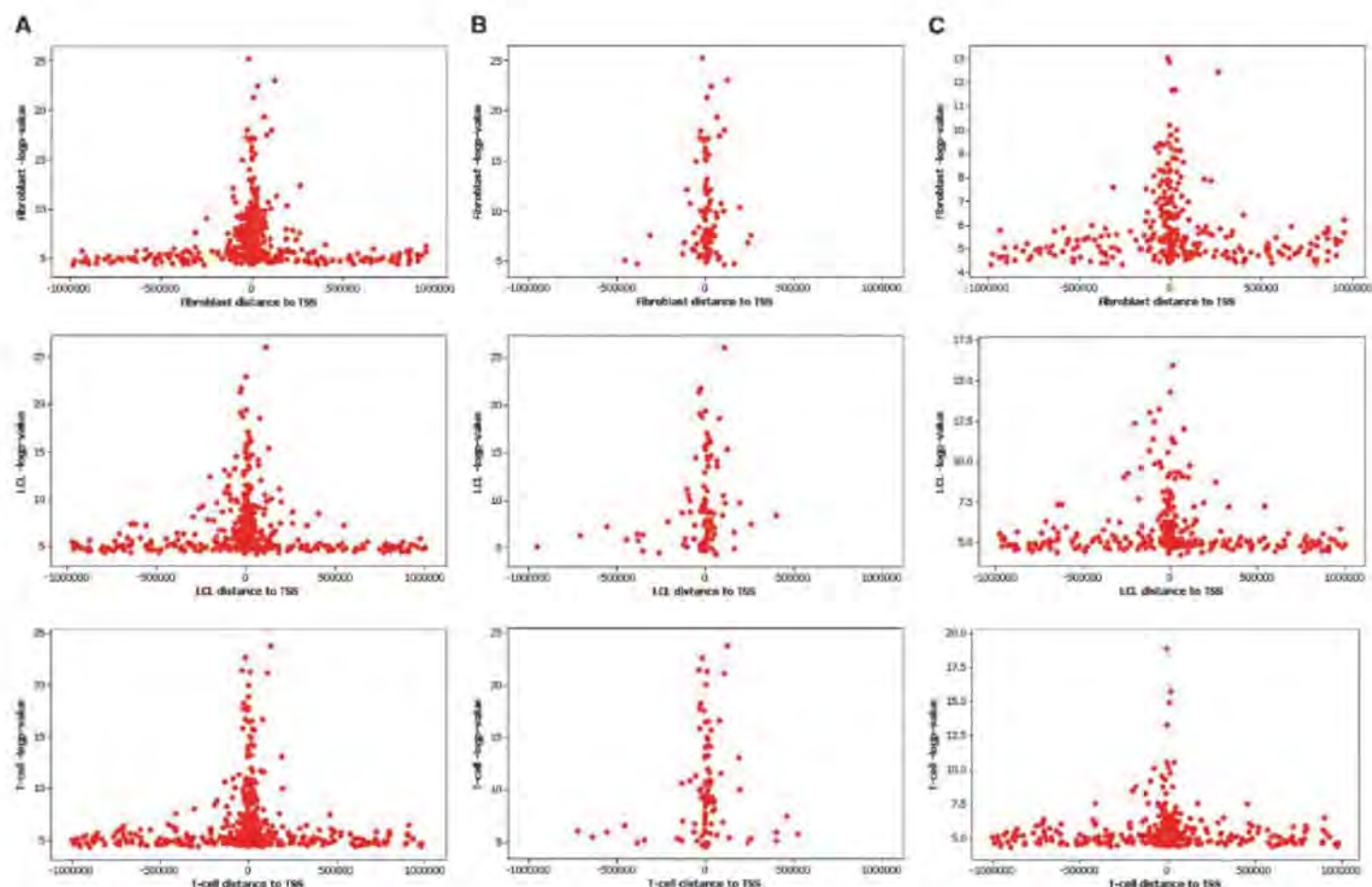


Fig. 3. Localization of independent cis eQTLs. Distance to TSS of (A) all independent cis eQTLs in each cell type (0.001 PT); (B) cis eQTLs shared in all three cell types (0.001 PT); and (C) cell type-specific cis eQTLs (0.001 PT).

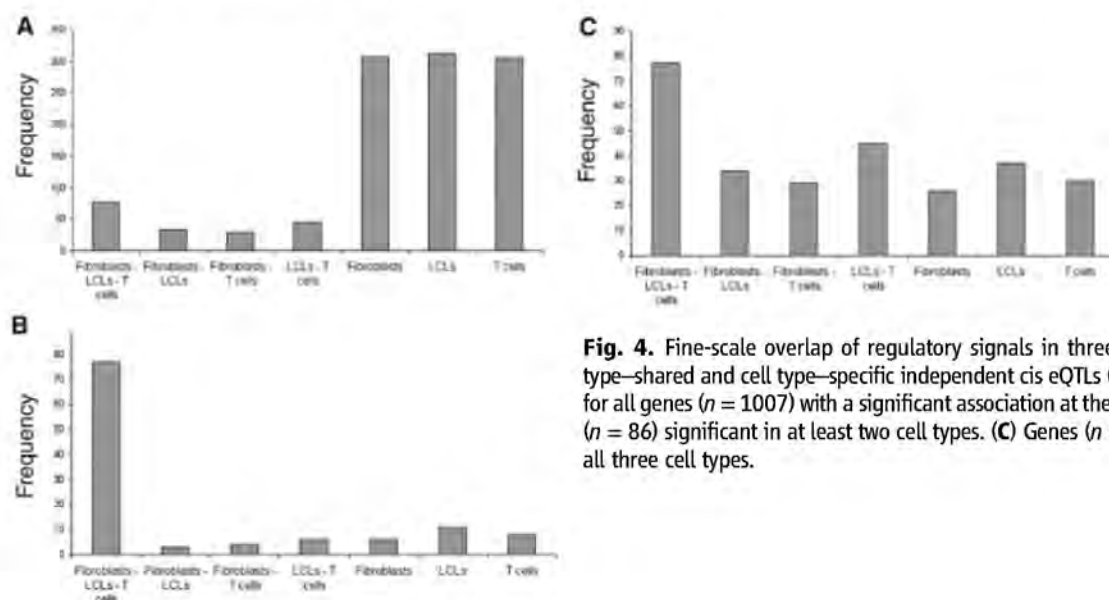


Fig. 4. Fine-scale overlap of regulatory signals in three cell types. (A) Cell type-shared and cell type-specific independent cis eQTLs (regulatory intervals) for all genes ($n = 1007$) with a significant association at the 0.001 PT. (B) Genes ($n = 86$) significant in at least two cell types. (C) Genes ($n = 206$) significant in all three cell types.

scripts per gene (Pearson's correlation coefficient = 0.049, $P = 0.117$ at 0.001 PT and Pearson's correlation coefficient = 0.105, $P < 0.0001$ at 0.01 PT). This suggests that regulatory complexity correlates with transcript complexity. Single-eQTL SNPs were also found to influence expression of multiple genes. At the 0.001 (and 0.01) PT, over 6% (19%) of eQTL SNPs were associated with the expression of more than one gene (fig. S10).

We used gene ontology (GO) (28) to compare the properties of cell type-specific and cell type-shared genes. We found an overrepresentation of functions linked to signal transducer activity, cell communication, development, behavior, cellular process, enzyme regulator activity, transcription regulator activity, and response to stimulus, which reflect processes likely to sculpt cell type-specific profiles. For eQTLs shared in all cell types, we found an overrepresentation of catalytic activity and transport properties (Fisher's exact test, $P < 0.05$) (table S5).

We have demonstrated that variants affecting gene regulation act predominantly in a cell type-specific manner, and even cell types as closely related as LCLs and T cells share only a minority of cis eQTLs. We estimate that 69 to 80% of regulatory variants are cell-type specific and that regulatory variant complexity correlates with transcript complexity, which implies that there are genotype-specific effects on alternative transcript choice. In

addition, cell type-specific eQTLs have smaller effects and tend to localize at greater distances from the TSS, recapitulating enhancer element distributions. The signal of cell-type specificity was shown to be primarily due to differential use of regulatory elements of genes that are expressed in almost all cell types. As more tissues are interrogated, we expect diminishing returns in discovery of eQTLs, and it is possible that there is a minimum set of informative tissues for the majority of regulatory variants. Our study highlights the need for extensive interrogation of regulatory variation in multiple cell types and tissues to elucidate their differential functional properties.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/1171418/DC1

Materials and Methods

Figs. S1 to S10

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References

27 March 2009; accepted 14 July 2009

Published online 30 July 2009;

10.1126/science.1171418

Include this information when citing this paper.

Rab35 Controls Actin Bundling by Recruiting Fascin as an Effector Protein

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Actin filaments are key components of the eukaryotic cytoskeleton that provide mechanical structure and generate forces during cell shape changes, growth, and migration. Actin filaments are dynamically assembled into higher-order structures at specified locations to regulate diverse functions. The Rab family of small guanine triphosphatases is evolutionarily conserved and mediates intracellular vesicle trafficking. We found that Rab35 regulates the assembly of actin filaments during bristle development in *Drosophila* and filopodia formation in cultured cells. These effects were mediated by the actin-bundling protein fascin, which directly associated with active Rab35. Targeting Rab35 to the outer mitochondrial membrane triggered actin recruitment, demonstrating a role for an intracellular trafficking protein in localized actin assembly.

A dynamic actin network is required for normal cell morphology, cell locomotion, and cytokinesis (1, 2). These processes require polymerization of globular actin monomers into filaments and bundling of the filaments under the control of actin-binding proteins (ABPs). Certain ABPs cross-link filamentous actin (F-actin)

into ordered parallel bundles that maintain the structural integrity of the cell and are structural components of filopodia, stereocilia, and microvilli (2–4). It is unclear how cells initiate the dynamic assembly of actin at the right times and places during development, physiological stresses, injury, and disease.

The importance of F-actin bundling during development is readily apparent during bristle formation in *Drosophila*. Bristles are mechanosensory organs found in genetically controlled locations on the cuticle. Their shapes and growth are dependent on actin bundles (5–7). The largest bristles, macrochaetae, are formed by a “shaft” cell that extrudes a cytoplasmic extension. This extension contains evenly distributed microtubules and F-actin bundles located just beneath the plasma membrane (Fig. 1, F and G). Bristle morphologies reflect the organi-

zation of actin bundles and can be used to study the regulation of actin in vivo.

Rab proteins constitute the largest subset of Ras-family small guanine triphosphatases (GTPases). Rab proteins control formation, motility, and docking of vesicles in specific trafficking pathways (8, 9) by recruiting specific effector proteins to different membrane compartments. Rab proteins are evolutionarily conserved: Each of the >70 types of mammalian Rab proteins is related to a particular *Drosophila* Rab protein. We tested all 31 *Drosophila* Rab GTPases systematically for their abilities to influence fly development, with the use of dominant negative (DN) mutant proteins produced in specific cell types (10). Rab activities are controlled by a cycle of associations with GTP or guanosine diphosphate (GDP). The DN mutants contained a Thr/Ser → Asn mutation that causes the proteins to bind preferentially to GDP and remain inactive. Overproduced DN proteins presumably tie up interacting proteins such as Rab exchange factors in nonproductive associations.

Only one of the 31 *Drosophila* Rab proteins had a strong effect on the actin cytoskeleton. Flies producing DN Rab35 (Rab35DN) in the peripheral nervous system (driven by *prospero-gal4*) exhibited unique and specific bristle morphology defects not seen with any other *Rab* DN gene. Rab35DN caused the development of adult bristles that had sharp bends, kinks, and forked ends in the thorax (Fig. 1, A to E) and other body regions including the head (fig. S6B). Bristles from Rab35DN-expressing flies, stained with the actin-binding dye phalloidin at 45 to 47 hours after puparium forma-

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tion, had a wavy, loose, and disconnected actin organization (Fig. 1H and fig. S2B) relative to the wild-type control (Fig. 1G and fig. S2A). Similar phenotypes are observed in mutants deficient for certain ABPs (5–7), which suggests that Rab35 may function as an ABP or through ABP(s). In the thoracic cuticle, production of Rab11DN or Rab5DN, which block Rab proteins that regulate endocytic trafficking, caused extensive defects in membrane growth and bristle distribution; these phenotypes are distinct from the Rab35DN effect (10). Rab35 was ubiquitously expressed, with transcripts and proteins especially abundant in the developing nervous system (fig. S1, C to H).

To test whether the defective-bristle actin phenotype was due to reduced Rab35 function, we expressed *UAS-Rab35* hairpin RNA interference (RNAi) in flies to reduce Rab35 mRNA (fig. S1A). The RNAi caused the same phenotypes as did Rab35DN (Fig. 1P), which confirmed that the DN protein was inhibiting the intended target. The bristle phenotype caused by Rab35RNAi was completely rescued upon expression of a mouse wild-type Rab35 gene (Fig. 1Q). In an otherwise wild-type genetic background, mouse Rab35DN caused the same phenotype as did fly Rab35DN (fig. S4C) in the peripheral nervous system. Thus, at least some functions of Rab35 protein are conserved from flies to mammals, an evolutionary span of ~500 million years.

Expressing wild-type Rab35 (Rab35WT) in cultured cells induced multiple filopodia-like cellular

extensions (Fig. 1J and figs. S3, A, B, C, D, and I, and S4E). No such effects were seen upon expression of Rab35DN (Fig. 1K and figs. S3, E to I, and S4F). Similarly, Rab35 induces peripheral processes in Jurkat T cells (11) and neurite outgrowth in PC12 and NIE-115 cells (12). The effect of extra Rab35 on cultured cells might reflect its role in vivo, allowing shaft cells to sprout protrusions during bristle development. Rab35DN, in contrast, stopped filopodia growth in vitro (Fig. 1K and figs. S3, E to H, and S4F) and caused defects in growing bristles in vivo (Fig. 1, B, D, and E). Ubiquitous Rab35DN expression driven by tubulin-gal4 caused lethality in embryos and, over a period of days, the death of cultured cells.

Treating *Drosophila* S2 cells with the actin polymerization inhibitor latrunculin A, but not with the microtubule-disrupting agent nocodazole, efficiently blocked the Rab35-driven morphology change (Fig. 1, L to N). Thus, Rab35 appeared to regulate actin assembly.

To explore how Rab35 influences the actin cytoskeleton, we set out to identify effector proteins that bind Rab35 directly. Rab effectors bind specific Rab proteins; binding is dependent on the Rab protein being in its GTP-bound, active state. The effectors have diverse functions in vesicle sorting, motor protein binding, vesicle trafficking, membrane fusion (13), and other roles yet to be defined. We used affinity chromatography to purify proteins that preferentially bind Rab35-GTP, which has been used to identify

other Rab effectors (14). Purified glutathione S-transferase (GST)-tagged Rab35WT protein was loaded with guanosine 5'-O-(3'-thiotriphosphate) (GTP- γ -S) or with GDP and incubated with bovine brain cytosolic extracts. Several proteins were found to bind Rab35-GTP- γ -S specifically (fig. S5A and table S1). Mass spectrometry revealed a prominent 55-kD polypeptide that bound Rab35-GTP- γ -S to be fascin. Myc-tagged Rab35 and FLAG-tagged fascin coimmunoprecipitated from cell extracts. Fascin bound more strongly to Rab35WT than to Rab35DN (Fig. 2A). Purified GST-Rab35 fusion protein bound fascin in vitro (Fig. 2B). Thus, Rab35 binds fascin directly. Fascin bound more strongly to Rab35WT preloaded with GTP- γ -S than to Rab35WT preloaded with GDP in immunoprecipitations of endogenous Rab35 from fly cells and in GST pull-downs (fig. S5, B and C), consistent with the identification of fascin as a Rab35 effector.

Fascin is an actin cross-linking protein that organizes F-actin into tightly packed parallel bundles in protruding (filopodia) and nonprotruding (microspike) structures at the leading edges of cells (15, 16). Higher than normal fascin levels have been associated with cancer cell migration, so the protein has been proposed as a marker for cancer diagnosis and a therapeutic target (17–19). Fascin is produced in many tissues and is especially abundant in the nervous system (20). In *Drosophila*, fascin mutants (called *singed*) are female sterile and have aberrant mechanosensory bristles (21, 22) due to

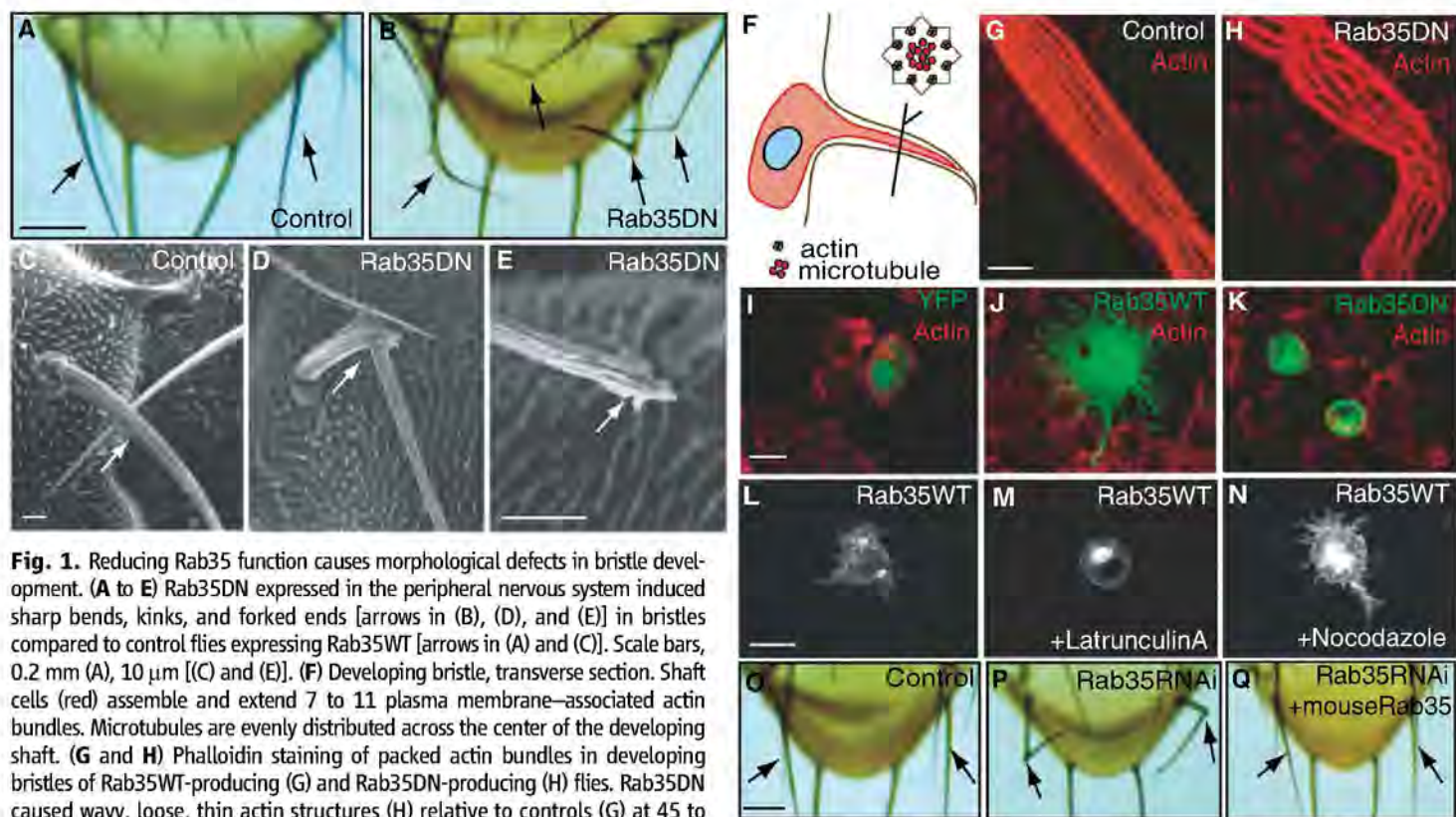


Fig. 1. Reducing Rab35 function causes morphological defects in bristle development. (A to E) Rab35DN expressed in the peripheral nervous system induced sharp bends, kinks, and forked ends [arrows in (B), (D), and (E)] in bristles compared to control flies expressing Rab35WT [arrows in (A) and (C)]. Scale bars, 0.2 mm (A), 10 μ m [(C) and (E)]. (F) Developing bristle, transverse section. Shaft cells (red) assemble and extend 7 to 11 plasma membrane-associated actin bundles. Microtubules are evenly distributed across the center of the developing shaft. (G and H) Phalloidin staining of packed actin bundles in developing bristles of Rab35WT-producing (G) and Rab35DN-producing (H) flies. Rab35DN caused wavy, loose, thin actin structures (H) relative to controls (G) at 45 to 47 hours after puparium formation. Texas Red–phalloidin staining detected the packed actin bundles. Scale bar, 10 μ m. (I to K) Relative to YFP-alone controls (I), YFP-Rab35WT (J) but not YFP-Rab35DN (K) induced filopodia-like membrane protrusions when expressed in S2 cells. Green, YFP proteins; red, phalloidin (filamentous actin). Scale bar, 5 μ m. (L to N) Latrunculin A administration completely suppressed the morphological changes caused by Rab35 (M) relative to vehicle control-treated cells (L). Treatment with nocodazole did not block the morphological changes (N). Scale bar, 5 μ m. (O to Q) Flies expressing Rab35RNAi in the peripheral nervous system had abnormal bristle morphology (P) relative to controls (O). A mouse Rab35WT transgene reversed this phenotype (Q). Scale bar, 0.2 mm.

dysfunctional actin structures. The *Drosophila* egg chamber is composed of a germline cyst surrounded by a somatic follicular epithelium. Each cyst consists of 15 nurse cells and one oocyte. Cortical cytoskeletal structures are required during late oogenesis when nurse cell cytoplasm is rapidly transferred

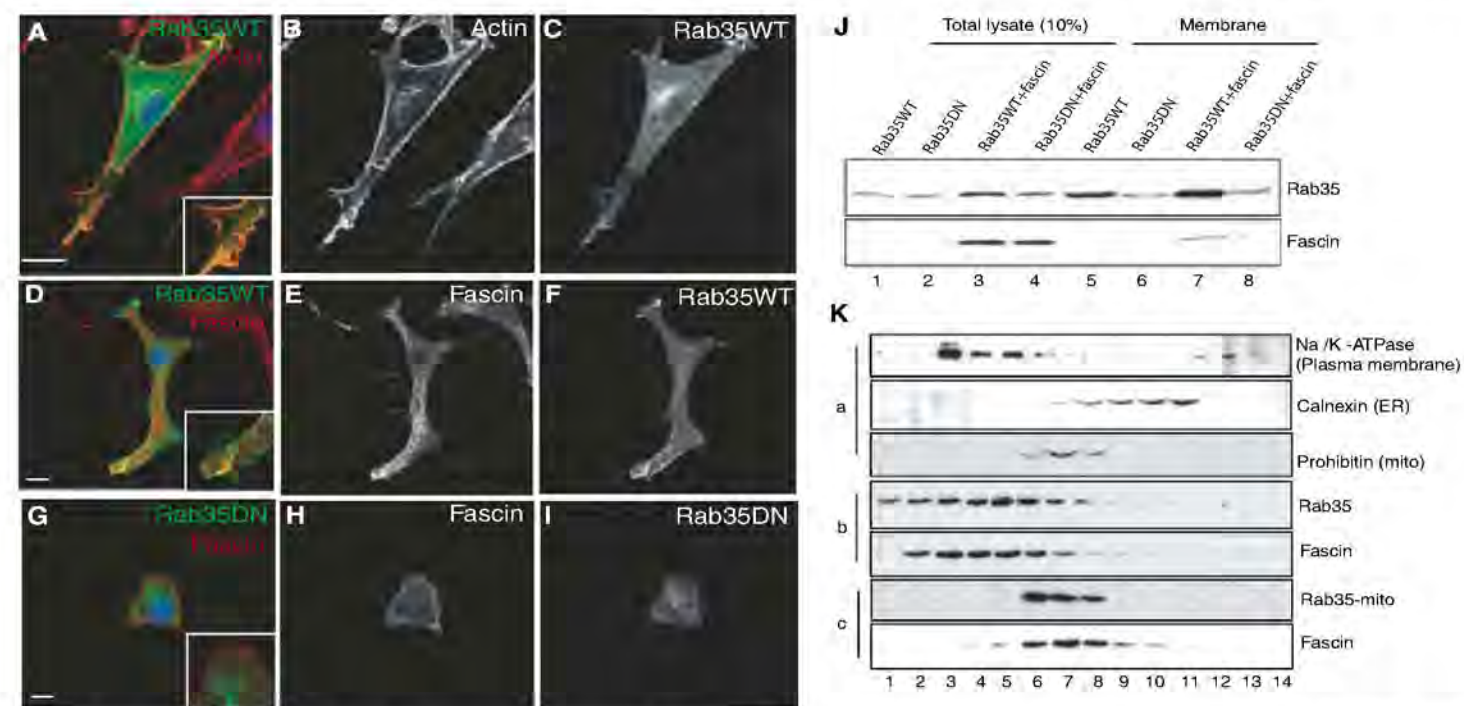
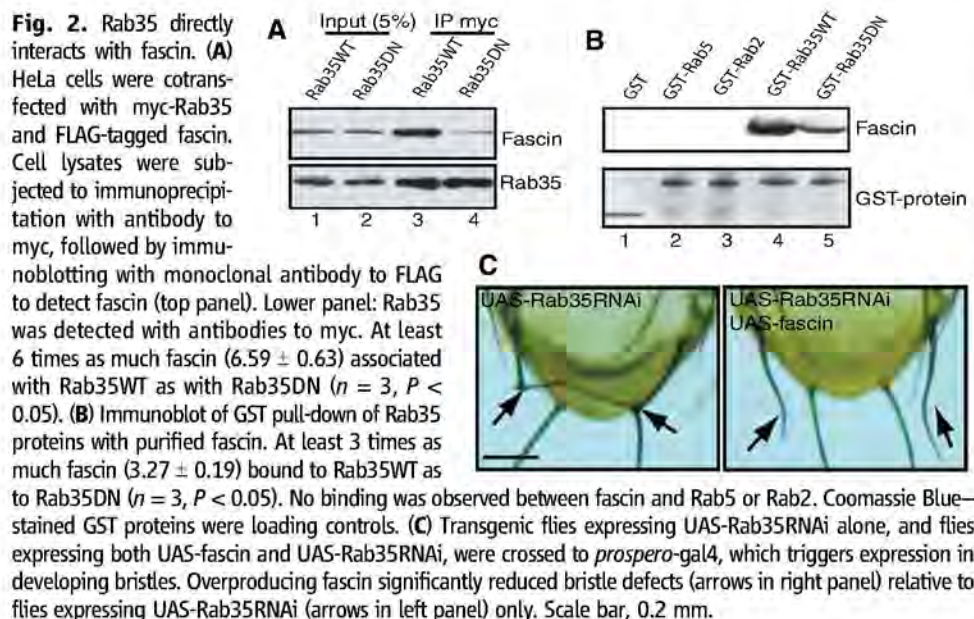
to the oocyte. The sterility phenotype of *singed* led us to examine the influence of Rab35DN in nurse cells (driven by tubulin-gal4 at 22°C) or follicle cells (driven by CY2-gal4). Both caused female sterility (94%). The interfering Rab35DN caused reduced ovarian actin levels relative to con-

trol flies, and ovary structure was abnormal (fig. S6, D, F, H, and J).

We tested whether the physical interaction between Rab35 and fascin was reflected in a genetic interaction. Rab35RNAi, produced in peripheral neurons, was used to damage bristle development. Altered bristle morphology was suppressed when extra fascin was supplied to the same cells (Fig. 2C). Increased fascin compensated for reduced Rab35 function, which suggests that fascin is at least one of the major proteins regulated by Rab35.

Purified GST-fascin and GST-Rab35 were mixed together or separately with purified non-muscle F-actin in vitro. Actin-bundling activity increased with fascin concentration (fig. S7, A and B). No effect of Rab35 on actin bundling was observed, alone or in combination with fascin (fig. S7, A and C). Thus, Rab35 has no discernible effect on actin bundling in vitro, but its association with fascin may be a means to control when or where actin is bundled in vivo.

Perhaps activated Rab35 recruits fascin to a subcellular location where fascin stimulates actin bundling. To explore this idea, we first examined the relative locations of Rab35 in different cell types and its association with fascin in mammalian cells. Rab35WT was enriched near the plasma membrane (Fig. 3, D to F, and figs. S6G and S8A)



level). More fascin was observed in the membrane fraction with Rab35WT (lane 7, lower panel) than with Rab35DN (lane 8, lower panel; 17.7% enrichment of membrane-associated fascin with Rab35WT normalized to the total fascin level; 10% of total protein lysates were loaded as controls (lanes 1 to 4). (K) Subcellular fractionation of NIH 3T3 cells. (a) Three markers were used to show the separation of different membrane compartments. (b) Cells treated as in (D) to (I) were fractionated. Rab35WT and fascin fractionated mainly with the plasma membrane marker Na^+ - and K^+ -dependent ATPase (enriched in fractions 3 to 5), but not with the endoplasmic reticulum marker calnexin or the mitochondria marker prohibitin. (c) Cells expressing YFP-Rab35-mito were also fractionated. An enrichment of fascin in the mitochondrial fraction was observed (fractions 6 to 8). From left to right, 2.5 to 25% iodixanol gradients.

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and colocalized with fascin at the leading edge of filopodia and within microspikes in lamellipodia (Fig. 3, D to F). Rab35DN caused severe alteration of cellular structure and accumulated to a greater extent in the cytosol than along the membrane (Fig. 3, G to I, and figs. S6H and S8B). In the presence of Rab35DN, less membrane-associated fascin was observed (Fig. 3, G to I). The membrane association of Rab35 and fascin was confirmed by a cell fractionation analysis; 33.4% of Rab35 fused to yellow fluorescent protein (YFP-Rab35) and 17.7% of fascin fractionated with the membrane fraction (Fig. 3J). The association of fascin with the membrane fraction was at least partially dependent on Rab35, because less Rab35 and less fascin associated with the membrane in cells where Rab35DN was expressed. Thus, Rab35 may bring fascin to the plasma membrane to influence subcortical actin structure and initiate filament bundling.

If fascin mediates the effects of Rab35, then Rab35-induced filopodia formation should be reduced when fascin function is blocked. Fascin was depleted in *Rab35*-expressing cells by introducing fascin small hairpin RNA (16). The interfering RNA significantly blocked filopodia

formation induced by Rab35 (fig. S9, B and C). Phosphorylation of fascin at Ser³⁹ is important for its actin-bundling activity and proper localization to filopodia (15, 16). We produced point mutants that mimic the active dephosphorylated (Ser³⁹ → Ala, S39A) or inactive phosphorylated (Ser³⁹ → Asp, S39D) forms of fascin (15, 16). Expression of tdTomato-tagged S39A or S39D fascin mutants in NIH 3T3 cells along with Rab35 had opposite effects on filopodia formation (figs. S9, D to F, and S10, A to I). The S39A mutant in combination with Rab35WT caused more protein to accumulate at the tip of cell extensions (figs. S9E and S10, D to F) relative to wild-type fascin plus Rab35WT, but no significant increase in the number of filopodia was observed (fig. S10J). In contrast, S39D in combination with Rab35 reduced the number of filopodia (fig. S10J). In these cells most of the Rab35WT protein remained near the plasma membrane (figs. S9F and S10, G to I). These results are consistent with a role for fascin as a downstream effector of Rab35 in filopodia formation.

We constructed a gene encoding a modified form of Rab35 targeted to the outer mitochon-

drial membrane, a location that normally has modest levels of fascin or assembled actin and no detectable Rab35 (Fig. 4, A to D). When Rab35DN was used in this experiment, the modified protein was on the surface of mitochondria (Fig. 4F). No discernible change in actin structure was observed in the vicinity of mitochondria (Fig. 4H and fig. S11, C and D). In contrast, when Rab35WT was brought to the mitochondrial outer membrane (Fig. 4, I to L), the mitochondria were consistently decorated with increased actin meshworks (Fig. 4L and fig. S11, A and B). As a control, Rab35WT targeted to mitochondria in the same manner did not cause actin assembly in the vicinity of mitochondria (Fig. 4, M to P). Fascin relocation to mitochondria was confirmed by cell fractionations. Mitochondrial enrichment of fascin was observed when Rab35WT-mito was produced (Fig. 3K). Thus, relocation of Rab35 can drive the location of actin assembly.

Our results show that a Rab35 effector protein, fascin, is able to stimulate local actin bundling and thus control bristle and filopodia formation (fig. S11E). The exact ways in which such a mechanism may be used probably vary among cell and tissue types. In cultured cells, active Rab35 recruits fascin to drive actin bundling at the leading edge of cell protrusions. During *Drosophila* bristle development, Rab35 may recruit fascin and induce actin bundling to initiate the cytoplasmic extension required for bristle extension. Inadequate Rab35 function leads to bends and kinks in the bristles.

Conflicting results about Rab35 function have been obtained from different cell types and models. Rab35RNAi revealed a function in cytokinesis in *Drosophila* S2 cells (23); in HeLa cells, Rab35 also plays a role in cytokinesis (23). No cytokinesis phenotype was observed in mutants of *Caenorhabditis elegans* Rab35, but Rab35 transports yolk receptors in oocytes (24). In HeLa-CIITA, a cell line in which major histocompatibility complex (MHC) class II is expressed, Rab35 regulates a recycling pathway in a clathrin-, AP2-, and dynamin-independent manner (25). In Jurkat T cells, Rab35-mediated recycling appears to be clathrin-dependent (11). In PC12 and N1E-115 cells, activated Rab35 stimulates neurite outgrowth via a Cdc42-dependent pathway (12). *Drosophila* Rab35, like mammalian Rab35, is found near the plasma membrane, on intracellular vesicles, and in the cytosol (23) (Fig. 3, A to C). This broad distribution contrasts with more discrete locations of other Rab proteins, including many we studied (10), which suggests that Rab35 may have diverse functions.

Interfering with Rab35 in living flies showed its importance for normal bristle morphology and its function in regulating actin assembly. The powerful influence of Rab35 on the cytoskeleton can now be at least partly explained by localization of fascin and its consequent influence on actin filament bundling.

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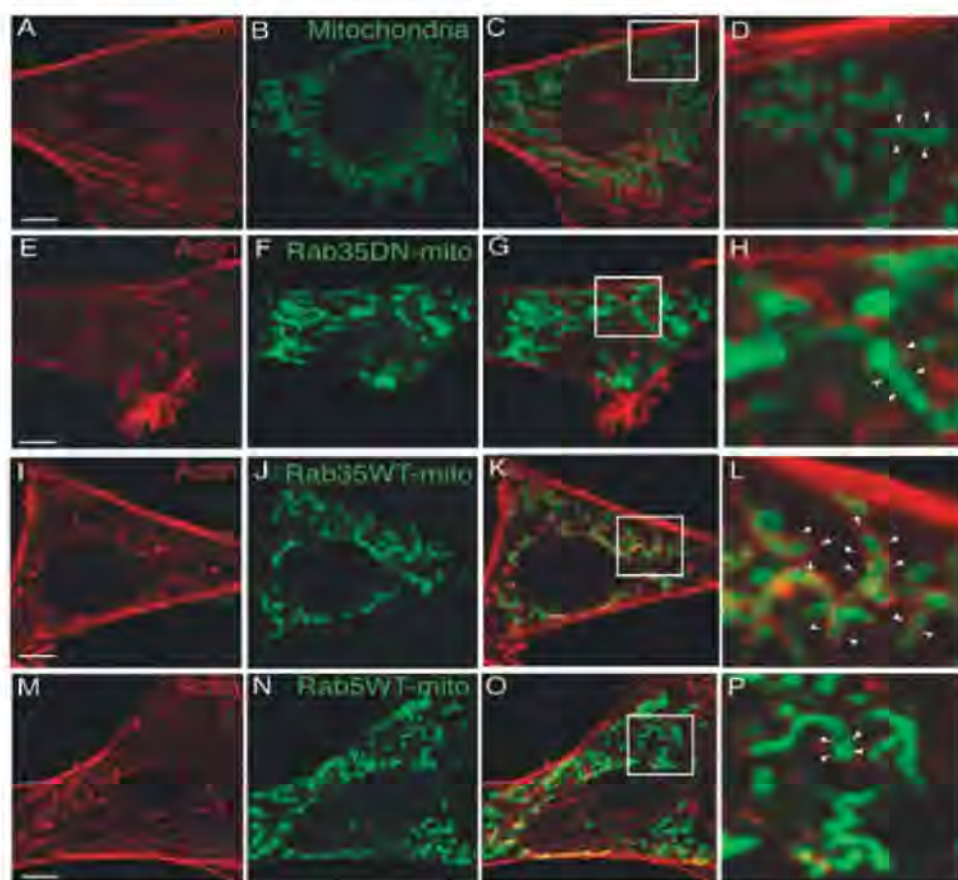


Fig. 4. Localized stimulation of actin bundling by Rab35 recruitment of fascin. (A to D) NIH 3T3 cells were stained with MitoTracker (Invitrogen; green) to show mitochondria and actin (red). Box in (C) is shown in (D) at higher magnification. Arrowheads indicate areas surrounding mitochondria. Scale bar, 5 μ m. (E to P) NIH 3T3 cells producing YFP-Rab35DN-mito, YFP-Rab35WT-mito, and YFP-Rab5-mito (all in green) were stained with phalloidin (red) to detect actin. In 20 to 30% of cells, localization of Rab35WT to mitochondria induced actin assembly in the vicinity of mitochondria (K). In parallel experiments in which Rab35DN and Rab5 were localized to mitochondria, no such enrichment of actin was observed [(G) and (O), respectively]. Boxes in (C), (G), (K), and (O) are shown in (D), (H), (L), and (P) at higher magnification. Arrowheads indicate areas surrounding mitochondria. Detectable accumulation of actin near mitochondria was observed only in (L). Scale bars, 5 μ m.

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26. We thank M. Fish for DNA injections; X. Huang, E. Bustamante, and C. Gauthier for help with initial experiments; Scott lab members for valuable discussion and comments; the Stanford Cell Sciences Imaging Facility

for assistance with scanning electron microscopy studies; and S. Pfeffer, A. Ghabrial, and R. Rohatgi for critical reading and comments on the manuscript. Supported by a Jane Coffin Childs Memorial Fund for Medical Research fellowship (J.Z.) and by the NIH National Technology Center for Networks and NIH Pathway grant U54 RR020843 (M.F. and M.B.). The research reported here was supported by the Howard Hughes Medical Institute. M.P.S. is an Investigator of the HHMI.

Supporting Online Material

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13 April 2009; accepted 10 July 2009
10.1126/science.1174921

Regulation of Histone Acetylation in the Nucleus by Sphingosine-1-Phosphate

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The pleiotropic lipid mediator sphingosine-1-phosphate (S1P) can act intracellularly independently of its cell surface receptors through unknown mechanisms. Sphingosine kinase 2 (SphK2), one of the isoenzymes that generates S1P, was associated with histone H3 and produced S1P that regulated histone acetylation. S1P specifically bound to the histone deacetylases HDAC1 and HDAC2 and inhibited their enzymatic activity, preventing the removal of acetyl groups from lysine residues within histone tails. SphK2 associated with HDAC1 and HDAC2 in repressor complexes and was selectively enriched at the promoters of the genes encoding the cyclin-dependent kinase inhibitor p21 or the transcriptional regulator c-fos, where it enhanced local histone H3 acetylation and transcription. Thus, HDACs are direct intracellular targets of S1P and link nuclear S1P to epigenetic regulation of gene expression.

Phospholipid and sphingolipid metabolites have established roles in signal transduction pathways initiated by activation of cell surface receptors. The recent identification of nuclear lipid metabolism has highlighted a new signaling paradigm for phospholipids. The best characterized of the intranuclear lipids are the inositol lipids that have critical roles in nuclear functions, such as pre-mRNA splicing, mRNA export, transcriptional regulation, and chromatin remodeling (1). Sphingomyelin has long been known to be a component of the nuclear matrix (2), but the possibility that sphingolipids are also metabolized within the nucleus has only recently emerged from observations that enzymes control-

ling sphingolipid metabolism, including neutral sphingomyelinase and ceramidase, are also present in the nucleus (3).

Sphingosine-1-phosphate (S1P) is a sphingolipid metabolite that regulates many cellular and physiological processes, including cell growth, survival, movement, angiogenesis, vascular maturation, immunity, and lymphocyte trafficking (4–6). Most of its actions are mediated by binding to a family of five heterotrimeric guanine nucleotide-binding protein (G protein)-coupled receptors, designated SIP_{1–5} (5). S1P may also function inside the cell independently of S1P receptors (4); however, direct intracellular targets of S1P have not been identified. Since the discovery that S1P is produced intracellularly by two closely related sphingosine kinase isoenzymes, SphK1 and SphK2, much has been learned about SphK1 and its functions, yet those of SphK2 remain enigmatic (7).

Because, in many cells, SphK2 is mainly localized to the nucleus or can shuttle between the cytosol and the nucleus in accordance with its nuclear localization and export signals (8), we sought to determine its function there (9). In human MCF-7 breast cancer cells, SphK2 is predominantly lo-

calized to the nucleus (10), where it is enzymatically active and can produce S1P from sphingosine (Fig. 1A). The nucleus contained high amounts of sphingosine (table S1), and SphK2 expression significantly increased nuclear abundance of S1P by sixfold and dihydro-S1P, which lacks the trans double bond at the 4 position, by twofold (Fig. 1A). Endogenous SphK2 was mainly associated with isolated chromatin and was not detected in the nucleoplasm (Fig. 1B). SphK2 was present in purified mononucleosomes fractionated by sucrose density gradient centrifugation, and its distribution paralleled that of native histones (fig. S1A). Thus, we tested whether SphK2 was associated with core histone proteins. Immunoprecipitation of proteins from nuclear extracts prepared from MCF-7 cells overexpressing SphK2 or SphK1 and subsequent Western blot analysis demonstrated that histone 3 (H3) was associated with SphK2 (Fig. 1C), but not with SphK1. H3 was also coimmunoprecipitated with catalytically inactive SphK2^{G212E} (in which glycine 212 is replaced by glutamic acid) (Fig. 1C), which does not increase nuclear S1P (4.4 ± 0.3 and 4.6 ± 0.6 pmol/mg for vector and SphK2^{G212E}, respectively); these findings suggest that enzymatic activity is not required for association of these two proteins. H3 from nuclear extracts also associated with histidine (His)-tagged SphK2 isolated with Ni²⁺-nitrilotriacetic acid (NTA)-agarose beads (fig. S1B). To further confirm the specificity of the physical interaction of SphK2 with H3, we measured in vitro association of Ni-NTA-agarose-bound His-tagged SphK2 or His-SphK1 with individually purified histones. Only H3, but not histones H4, H2B, or H2A, bound to SphK2 (fig. S1C). In contrast, none of the purified histones interacted with SphK1 (fig. S1C).

We noticed that expression of SphK2 increased acetylation of lysine 9 of H3 (H3-K9), lysine 5 of histone H4 (H4-K5), and lysine 12 of histone H2B (H2B-K12) (Fig. 2A), without affecting acetylation of other lysines or acetylation of histone H2A. In contrast, expression of catalytically inactive SphK2^{G212E} (Fig. 2A) or SphK1 did not influence acetylation of any residues examined. Although catalytically inactive SphK2^{G212E} also bound to

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H3 (Fig. 1C), it did not affect its acetylation status (Fig. 2A), which correlated with the formation of S1P in the nucleus with histone acetylation. Addition of S1P or dihydro-S1P, which can both be produced intranuclearly by SphK2 (Fig. 2D), to isolated nuclei also increased specific acetylations of the same lysine residues on histones H3, H4, and H2B without causing acetylation of H2A (Fig. 2B).

All these results indicate that SphK2 in the nucleus regulates histone acetylation. Depletion of SphK2 with small interfering RNA (siRNA) (Fig. 2C) reduced nuclear but not cytoplasmic amounts of S1P and dihydro-S1P (Fig. 2D), consistent with the specificity of SphK2 for both sphingosine and dihydrosphingosine (11). As expected from its cytosolic localization, siRNA tar-

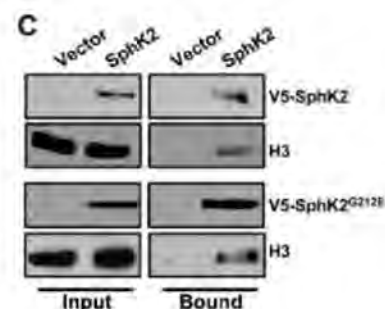
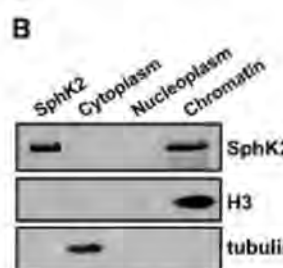
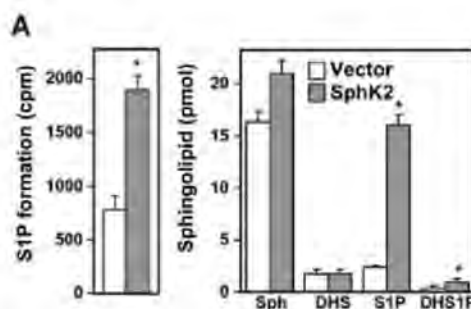
geted to SphK1 (siSphK1) only reduced amounts of S1P in the cytoplasm (Fig. 2D). siSphK2 specifically decreased acetylation of H3-K9, H4-K5, and H2B-K12, without affecting acetylation of other lysines or acetylation of H2A (Fig. 2C). In contrast, depletion of SphK1 had no significant effect on acetylation of these histone residues (Fig. 2C). Similar results were obtained with siRNAs targeted to different sequences in SphK2. Addition of S1P to isolated nuclei reversed the decreased acetylation of H3-K9, H4-K5, and H2B-K12 induced by depletion of SphK2 (fig. S2A). siRNA-mediated depletion of endogenous SphK2 (fig. S2B), but not that of overexpressed V5-SphK2 (fig. S2C), decreased acetylation of particular histone residues (fig. S2C), which could be reversed by cotransfection with a siRNA-insensitive V5-

SphK2 construct (fig. S2C). Together, these observations indicate that S1P produced in the nucleus by endogenous SphK2 acts there to regulate histone acetylation.

Lysine acetylation of histones is a critical component of an epigenetic indexing system demarcating transcriptionally active chromatin domains. Histone acetyltransferases (HATs) and histone deacetylases (HDACs) maintain the delicate, dynamic equilibrium in acetylation levels of nucleosomal histones (12, 13). Neither S1P nor dihydro-S1P increased HAT activity in HeLa cell nuclear extracts, nor did overexpression of SphK2 (fig. S3). In sharp contrast, S1P and dihydro-S1P inhibited HDAC activity in nuclear extracts by 50%, compared with 95% inhibition by the HDAC inhibitor trichostatin A (TSA) (fig. S4A). Con-

Fig. 1. Presence of

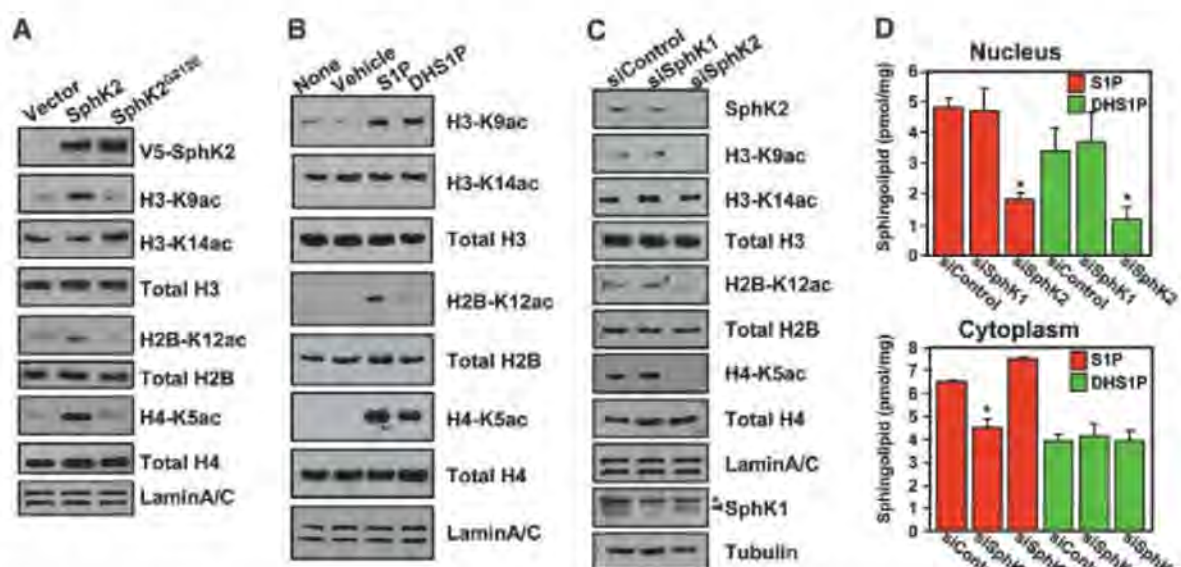
SphK2 in mononucleosomes and physical association with histone H3. (A) Nuclei were isolated from MCF-7 cells transfected with vector (white bar) or SphK2 (gray bar) and incubated with [3 H]sphingosine (1.5 μ M) and ATP (1 mM) for 30 min. Formation of [3 H]S1P was determined by differential extraction (22). Abundance of sphingoid bases sphingosine (Sph) and dihydrosphingosine (DHS), and their phosphorylated products, S1P and dihydro-S1P (DHS1P), in nuclei of vector and SphK2-expressing MCF-7 cells (7×10^6) were determined by liquid chromatography electrospray ionization mass spectrometry (LC-ESI-MS/MS). The data are averages of triplicate determinations and are expressed as picomoles of lipid $\pm$ SD. Asterisks indicate statistically significant differences ($P < 0.05$ by Student's t test) relative to vector transfectants. (B) Association of SphK2 with chromatin. Equal amounts of cytosol, nucleoplasm, and chromatin frac-



tions from MCF-7 cells were separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotted with SphK2-specific antibody. Antibodies against H3 and tubulin were used as markers for chromatin and cytosol, respectively. Lysate from MCF-7 cells overexpressing SphK2 was included as a positive control (leftmost lane). (C) Association of SphK2 and histone H3. Proteins of nuclear extracts from MCF-7 cells transfected with vector, V5-SphK2, or catalytically inactive V5-SphK2^{G212E} were immunoprecipitated with V5-specific antibody, separated by SDS-PAGE, and probed with antibodies against V5 or H3.

Fig. 2. SphK2 and S1P enhance histone acetylation.

(A) Histone acetylation in nuclear extracts from MCF-7 cells transfected with vector, V5-SphK2, or catalytically inactive V5-SphK2^{G212E}. Acetylation was detected by immunoblotting with antibodies to specific histone acetylation sites as indicated. (B) Effect of S1P and dihydro-S1P on histone acetylation. Purified nuclei from MCF-7 cells were treated for 5 min without (none) or with vehicle, 1 μ M S1P, or 1 μ M dihydro-S1P, and histone acetylation was examined by Western blotting. (C and D) Effect of depletion of SphKs. MCF-7 cells were transfected with nontargeting control siRNA, or siRNA targeted to SphK1 or SphK2. (C) Proteins from nuclear extracts were immunoblotted as indicated. Depletion of SphK1 and SphK2 was confirmed by immunoblotting with SphK1-specific and SphK2-specific antibodies, respectively.



Asterisk (*) indicates nonspecific band. (D) Amounts of S1P and dihydro-S1P in purified nuclei and cytoplasm quantified by LC-ESI-MS/MS. Asterisks indicate statistically significant differences ($P < 0.05$ by Student's t test) relative to control siRNA.

versely, siSphK2, which reduced SphK2 expression by 70% in HeLa cells without affecting SphK1 expression (fig. S4C), enhanced HDAC activity (fig. S4B). This increased HDAC activity was not due to increased abundance of HDAC1 or HDAC2 (fig. S4D). We also assessed the effect of S1P on enzymatic activity of endogenous HDACs. S1P inhibited the enzymatic activity of immunoprecipitated HDAC1 (Fig. 3A) and HDAC2 (fig. S4E) almost as effectively as did TSA. Both S1P and dihydro-S1P also inhibited recombinant HDAC1 (Fig. 3B) and HDAC2 (fig. S4F). However, neither sphingosine nor lysophosphatidic acid (LPA), another bioactive lysophospholipid structurally related to S1P, had significant effects on HDAC1 (Fig. 3B) or HDAC2 activity (fig. S4F). Thus, S1P and dihydro-S1P appear to be endogenous inhibitors of HDAC. To provide further evidence that HDACs are targets for S1P, we examined binding of endogenous HDACs in nuclear extracts to S1P immobilized on agarose beads. Both HDAC1 and HDAC2 were associated with matrices carrying S1P but not by control or LPA matrices (Fig. 3C). In contrast, other class I HDACs (HDAC3, HDAC8), class IIa HDACs (HDAC4, HDAC5, HDAC7), class IIb HDAC (HDAC6), and the nicotinamide adenine dinucleotide (NAD⁺)-dependent protein deacetylase SIRT1 did not bind to S1P agarose beads (fig. S5). ³²P-labeled S1P specifically bound to either His-tagged

HDAC1 or HDAC2 immobilized on Ni-NTA-agarose beads and was displaced by excess unlabeled S1P (fig. S6). Modeling of S1P into the active site of the HDAC1 homolog from the hyperthermophilic bacterium *Aquifex aeolicus* (14) revealed that it docks well (fig. S7), with a predicted binding energy comparable to that of suberoylanilide hydroxamic acid (SAHA) (fig. S7), a potent inhibitor of HDACs. Indeed, both SAHA and TSA completely displaced bound [³²P]S1P from HDAC1 and decreased binding to HDAC2 to a similar extent, as did excess unlabeled S1P (fig. S6). To determine whether endogenous S1P is bound to nuclear HDAC1, we measured the abundance of sphingolipids in HDAC1 immunoprecipitates from nuclear extracts by mass spectrometry. Of all the sphingolipids present in the nucleus, only S1P was bound to HDAC1 (Fig. 3D and table S1). Thus, S1P can bind to the active site of HDAC1 and HDAC2 and inhibit their activity.

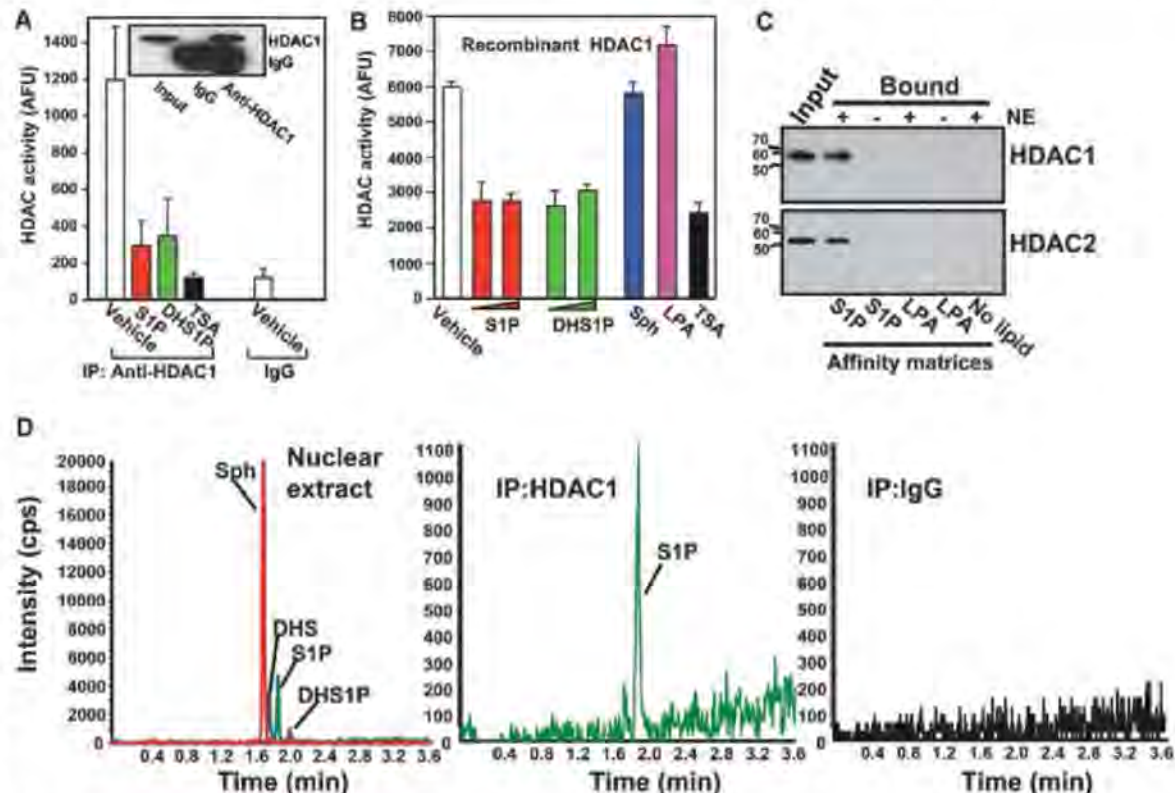
HDAC1 and HDAC2 are catalytically active components of distinct multiprotein co-repressor complexes that include Sin3 and NuRD. Overexpressed SphK2 associated with proteins of these co-repressor complexes, mSin3A and MBD2/3, and with HDAC1 and HDAC2 (fig. S8A). Endogenous SphK2 also associated with HDAC1 and HDAC2 and interacted with histone H3 (fig. S8B). These results place SphK2, the enzyme that produces S1P in the nucleus, in close prox-

imity to its potential nuclear targets. To determine whether nuclear S1P levels are regulated in response to external signals and whether HDACs sense these changes, we treated cells with phorbol 12-myristate 13-acetate (PMA), an activator of protein kinase C, which enhances phosphorylation and catalytic activity of SphK2 (15). Indeed, PMA increased amounts of S1P in the nucleus by more than twofold within 5 min (fig. S8C) and also rapidly enhanced colocalization of SphK2 with HDAC1 (fig. S9). Prolonged treatment with PMA induced nuclear export of SphK2 (8) (fig. S9), which ensured transient inhibition of HDACs.

A common response to inhibition or depletion of HDAC1 or HDAC2 is the induction of cyclin-dependent kinase inhibitor p21 independently of the p53 tumor suppressor protein (16–19). In agreement with possible involvement of SphK2 in p53-independent expression of p21 (10), silencing SphK2 in MCF-7 cells reduced basal and PMA-induced accumulation of p21 protein (fig. S10A) and mRNA (fig. S10B). Overexpression of SphK2, but not catalytically inactive SphK2, enhanced PMA-stimulated transcription of a reporter construct containing the p21 promoter (fig. S10C), and its depletion reduced it (fig. S10D). Overexpression of SphK2 increased acetylation of histone H3-K9 associated with the proximal p21 promoter and further enhanced the response to PMA (Fig. 4A), in accordance with

Fig. 3. S1P binds and inhibits HDAC1. (A and B)

Effect of S1P and dihydro-S1P on HDAC1 activity. HeLa cell nuclear extracts were immunoprecipitated with control IgG or HDAC1-specific antibody. Immunoprecipitates were washed, and HDAC activity was measured in the presence of vehicle, 1 μ M S1P, 1 μ M dihydro-S1P (DHS1P), or 1 μ M trichostatin A (TSA). HDAC activities are averages of triplicate determinations $\pm$ SD and expressed as arbitrary fluorescence units (AFU). (Inset) Immunoprecipitated proteins were separated by SDS-PAGE and analyzed by Western blotting with HDAC1-specific antibody. (B) Activity of recombinant HDAC1 (250 ng) determined in the absence or presence of two concentrations of S1P or dihydro-S1P (0.5 or 5 μ M), sphingosine (5 μ M), LPA (5 μ M), or TSA (1 μ M). (C) Association of HDAC with immobilized S1P. Nuclear extracts (NE) from MCF-7 cells were incubated with control (no lipid), LPA, or S1P affinity matrices (as indicated), then washed and bound proteins were resolved by SDS-PAGE and analyzed by Western blotting with antibodies against HDAC1 or HDAC2. (D) Detection of sphingoid bases. Extracted ion chromatogram for



LC-MS/MS reverse-phased separation of sphingoid bases in nuclear extracts (left), or HDAC1-specific antibody (center), and control IgG immunoprecipitates (right). Quantification of sphingosine (Sph) and dihydrosphingosine (DHS), and their phosphorylated products, S1P and dihydro-S1P (DHS1P), is shown in table S1.

the participation of SphK2 in PMA-induced expression of *p21* (fig. S10, A to D). Chromatin immunoprecipitation (ChIP) analysis also revealed that, like HDAC1, SphK2 is also associated with the proximal *p21* promoter (Fig. 4A). Depletion of SphK2 suppressed PMA-induced acetylation of histone H3 at the *p21* promoter (Fig. 4C), which further supported the notion that SphK2 regulates HDAC1-dependent deacetylation of histone H3 and repression of *p21* gene transcription.

We also examined another target gene that shows increased transcription in cells treated with PMA, *c-fos* (12, 20). SphK2 depletion compromised the induction of endogenous *c-fos* expression by PMA (fig. S11A) and blocked enhanced *c-fos* promoter activity (fig. S11B). Overexpression of SphK2, but not that of catalytically inactive SphK2, increased PMA-induced expression of *c-fos* mRNA (fig. S11C) and enhanced *c-fos* promoter activity (fig. S11D). Finally, ChIP analysis confirmed the presence of SphK2 on the

c-fos promoter and demonstrated that overexpression of SphK2 (but not that of the mutant SphK2 that cannot produce S1P) enhanced H3 acetylation at the *c-fos* promoter (Fig. 4B). Conversely, down-regulation of SphK2 decreased acetylation of histone H3 at the *c-fos* promoter in response to PMA (Fig. 4D).

We conclude that HDACs appear to be intracellular targets of S1P. Our data reveal a new paradigm of S1P signaling produced by nuclear sphingolipid metabolism, suggesting an important function for SphK2 in the nucleus. Abundance of nuclear S1P may influence specific and contextual chromatin states that impact gene transcription (Fig. 4E). HDACs have emerged as key targets to reverse aberrant epigenetic changes associated with human diseases (18, 21), such as cancer. S1P may therefore influence the delicate balance and dynamic turnover of histone acetylation and the transcription of target genes, linking them to epigenetic regulation in response to environmental signals.

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23. This work was supported by grants from the NIH R37GM043880 and R01CA1774 (S.S.). G.S. was supported by National Research Service Award F30NS058008. S.M. was supported by the NIMH Intramural Research Program.

Supporting Online Material

www.sciencemag.org/cgi/content/full/325/5945/1254/DC1
Materials and Methods

Figs. S1 to S11

Table S1

References

22 May 2009; accepted 21 July 2009

10.1126/science.1176709

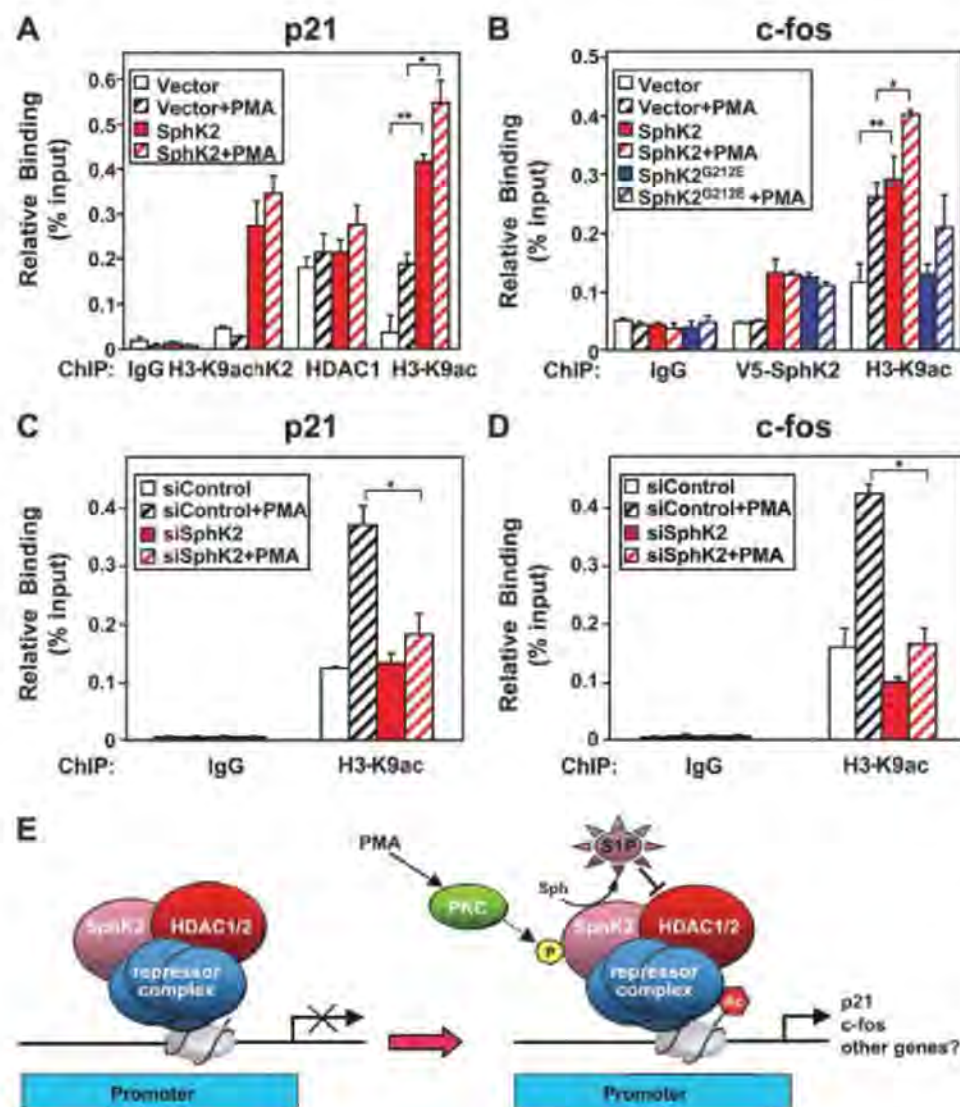


Fig. 4. SphK2 binding to *p21* and *c-fos* promoters enhances acetylation of histone H3. (A and B) MCF-7 cells transfected with vector, V5-SphK2, or V5-SphK2^{G212E} were treated with vehicle or PMA (100 nM, hatched bars) for 3 hours (A) or 30 min (B) and subjected to ChIP analyses with antibodies to V5, HDAC1, H3-K9ac, or normal rabbit IgG, as indicated. The precipitated DNA was analyzed by real-time PCR with primers amplifying the core promoter sequence of the *p21* and *c-fos* genes. Relative binding to the promoter is expressed as the percentage of input. Data are means $\pm$ SD. ** $P < 0.01$, relative to vector transfectants, * $P < 0.01$, relative to PMA-treated vector transfectants, by Student's *t* test. (C and D) MCF-7 cells transfected with control siRNA or siSphK2 were treated with vehicle or PMA (100 nM, hatched bars) for 3 hours (C) or 30 min (D) and subjected to ChIP analyses with antibody against H3-K9ac or with normal rabbit IgG. * $P < 0.01$, relative to PMA-treated siControl. (E) Model for regulation of histone acetylation and gene transcription by nuclear SphK2 and S1P. PMA stimulates nuclear SphK2, which is associated with specific promoter regions, such as those for *p21* and *c-fos* genes, and increases production of S1P. S1P in turn inhibits HDAC1 and HDAC2, which results in increased acetylation (Ac) of histone(s) and leads to enhanced gene transcription.

Perineuronal Nets Protect Fear Memories from Erasure

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In adult animals, fear conditioning induces a permanent memory that is resilient to erasure by extinction. In contrast, during early postnatal development, extinction of conditioned fear leads to memory erasure, suggesting that fear memories are actively protected in adults. We show here that this protection is conferred by extracellular matrix chondroitin sulfate proteoglycans (CSPGs) in the amygdala. The organization of CSPGs into perineuronal nets (PNNs) coincided with the developmental switch in fear memory resilience. In adults, degradation of PNNs by chondroitinase ABC specifically rendered subsequently acquired fear memories susceptible to erasure. This result indicates that intact PNNs mediate the formation of erasure-resistant fear memories and identifies a molecular mechanism closing a postnatal critical period during which traumatic memories can be erased by extinction.

Pairing an initially neutral stimulus (conditioned stimulus; CS) with an aversive stimulus (unconditioned stimulus; US) leads to the formation of a robust and long-lasting fear memory (1). In rats, such memories can last for the entire lifetime (2). Inhibition of conditioned fear responses can be achieved by repeated exposure to the CS in the absence of the US, a process called extinction (3). Unlike fear conditioning, in adult animals fear extinction is neither robust nor permanent. After extinction training, conditioned fear responses can recover spontaneously, after reexposure to the US (reinstatement), or in response to a context shift (renewal) (3–5). This strongly indicates that fear extinction does not erase previously acquired fear memories, but involves new learning eventually inhibiting conditioned fear behavior.

In stark contrast to adult animals, rats younger than 3 weeks do not exhibit reinstatement or context-dependent renewal of conditioned fear responses (6, 7). During early postnatal development, extinction thus appears to be permanent and has been suggested to reflect an unlearning process that leads to the erasure of previously conditioned fear memories (8). Both in adults and in young animals, fear extinction depends on the amygdala (3, 8). However, the neuronal mechanisms underlying the developmental regulation of fear extinction are not known.

Developmental regulation of brain plasticity is much better understood in sensory systems, such as the visual cortex. During the first few weeks of postnatal development, the so-called critical period, monocular sensory deprivation leads to long-lasting functional and structural changes (9). The absence of perineuronal nets

(PNNs), a highly organized form of chondroitin sulfate proteoglycans (CSPG)-containing extracellular matrix (10), is considered to be a key permissive factor that allows the induction of ocular dominance plasticity during the critical period. The assembly of PNNs around parv-

albumin (PV)-expressing inhibitory interneurons is thought to contribute to critical-period closure (11). Consistent with this notion, the degradation of PNNs in adults reenables the induction of ocular dominance plasticity (11). On the basis of these observations in sensory systems, we hypothesized that related developmental plasticity mechanisms may influence emotional learning processes in juveniles. Here, we examined whether in the amygdala, postnatal maturation of PNNs may underlie the developmental switch in fear extinction regulation.

We first quantified the time course of PNN formation in the basolateral amygdala (BLA) during the first 4 postnatal weeks (Fig. 1, A and B). The number of detectable *Wisteria floribunda* agglutinin (WFA)-stained PNNs increased markedly until postnatal day 28 (P28), when they were comparable to adult levels (Fig. 1B). Notably, the largest increase was observed between P16 and P21 (Fig. 1, A and B), the age around which the switch in the extinction phenotype occurs in rats (8).

We therefore compared spontaneous recovery and context-dependent renewal of extinguished

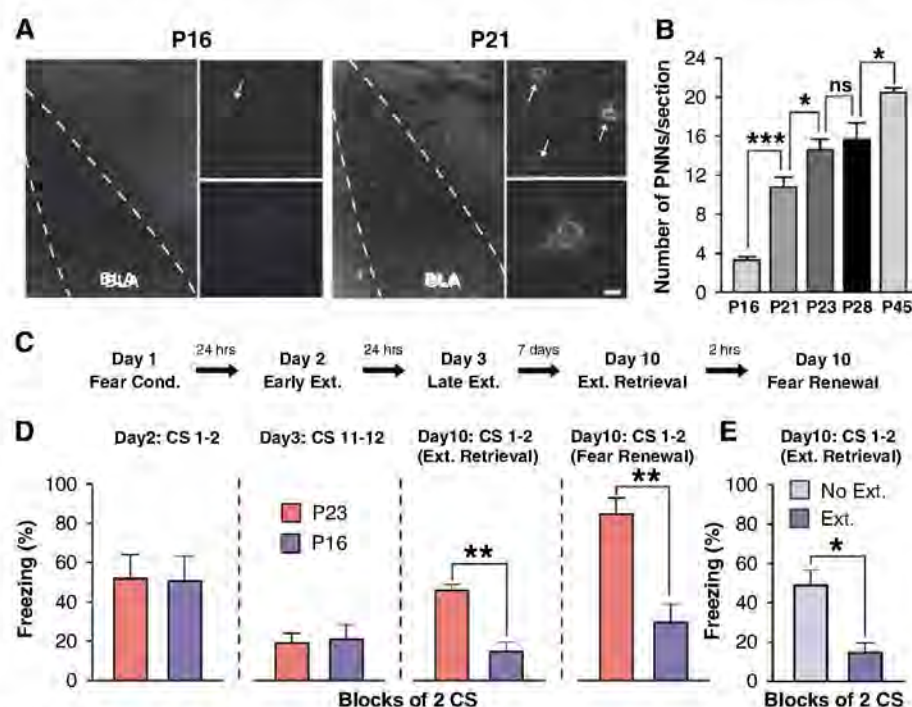


Fig. 1. Coincident developmental switch in PNN formation and in the susceptibility of fear memories to erasure. (A) Left panels: Overview of WFA staining in the BLA of P16 and P21 mice. Right panels: Higher-power images of PNNs in the BLA of the same age groups. Scale bar, 200 μ m (left panels), 30 μ m (top right panels), 10 μ m (bottom right panels). (B) The number of WFA-positive PNNs in the BLA increases throughout postnatal development [one-way analysis of variance (ANOVA): $F_{(3,19)} = 23.4$, $P < 0.001$]. The largest increase is seen between P16 and P21 (Student-Newman-Keuls post-hoc tests). (C) Experimental protocol. Fear cond.: fear conditioning, Ext.: extinction. (D) One week after extinction training, comparison of freezing levels in mice conditioned at P16 ($n = 5$) or P23 ($n = 4$) revealed significant spontaneous recovery and context-dependent renewal in the P23, but not in the P16 group (percent of time spent freezing, extinction retrieval: P16: 14.6 ± 4.9 , P23: 45.8 ± 3.2 , $P < 0.01$; renewal: P16: 29.6 ± 9.4 , P23: 84.8 ± 8.1 ; $P < 0.01$, two-tailed unpaired t test). In P23 mice, freezing levels during fear renewal were significantly greater than during extinction retrieval ($P < 0.05$, two-tailed paired t test). (E) In the absence of extinction training, P16 animals ($n = 7$) showed stable fear memory 10 days after conditioning (percent of time spent freezing: P16 No Ext: 48.7 ± 7.9 , P16 Ext: 14.6 ± 4.9 ; $P < 0.05$, two-tailed unpaired t test). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; ns, not significant.

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fear responses in young mice conditioned either before (P16) or after (P23) the age of 3 weeks (Fig. 1C). After fear conditioning, both groups exhibited equal levels of freezing behavior, which was strongly reduced by subsequent extinction training in a different context (Fig. 1D). Seven days after extinction training, mice were reexposed to the CS in both contexts. Mice conditioned at P23 exhibited significant spontaneous recovery and renewal when tested in the extinction context, or in the fear conditioning context, respectively (Fig. 1D). In contrast, in mice conditioned at P16, freezing levels did not increase compared with those measured at the end of extinction training, independent of the context in which they were exposed to the CS (Fig. 1D). The absence of spontaneous recovery and renewal did not reflect a passive loss of fear memory, because mice conditioned at P16 retained a stable fear memory for 10 days (Fig. 1E).

If PNNs in the BLA were causally related to the protection of fear memories from erasure by extinction, we hypothesized that it should be possible to convert the extinction phenotype of adult mice into a juvenile one by acutely destroying PNNs in adults. To test this, we locally injected the CSPG-degrading enzyme chondroitinase ABC

(ChABC) (11, 12) into the BLA of 3-month-old mice (fig. S1). Twenty-four hours after ChABC injection, no PNNs could be detected in the BLA (Fig. 2A). Mice fear conditioned 24 hours after ChABC injection (Fig. 2B) exhibited normal freezing levels compared with vehicle-injected controls when exposed to the CS 24 hours after conditioning (Fig. 2C). Moreover, both groups exhibited low freezing levels when exposed to an explicitly unpaired CS, indicating that conditioned freezing reflected CS-US associations rather than non-associative sensitization processes (fig. S2).

After extinction training, freezing levels were equally reduced in ChABC- and vehicle-injected mice (Fig. 2C). However, when tested 7 or 28 days later, ChABC-injected mice, like juvenile mice conditioned at P16, exhibited a complete lack of spontaneous recovery and context-dependent renewal (Fig. 2C and figs. S3 and S4). Even though cued (CS-induced) fear behavior was strongly compromised, contextual fear memory was normal in ChABC-injected animals (Fig. 2C), which demonstrates that the absence of fear renewal cannot be explained by a deficit in context discrimination or by a lack of attention. Because contextual fear was not extinguished, this indicated that extinction training was necessary

for the ChABC-induced permanent loss of conditioned fear behavior. To examine the time course of extinction-induced behavioral changes in ChABC-injected mice, we analyzed freezing levels during extinction training. Whereas a total of 24 CSs in two extinction training sessions distributed over 2 days was necessary to achieve full extinction of conditioned fear responses in control animals (Fig. 2D) (13), exposing ChABC-injected mice to just three CSs substantially decreased freezing levels, and after seven CS presentations, freezing behavior was already at baseline levels (Fig. 2D). We also examined the time course of extinction learning in juvenile mice conditioned at P16 or P23. Freezing behavior during extinction training was much more variable in juvenile animals compared with adults, but there was no significant group difference in the time course of extinction learning between juvenile mice conditioned at P16 or P23. The lack of accelerated within-session extinction in P16 mice could be related to the presence of diffuse WFA staining, which was not observed in adult animals treated with ChABC. Thus, in the absence of PNNs, extinction training triggers a rapid process that results in the acute and permanent loss of conditioned fear behavior, suggesting that PNNs in the amygdala prevent unlearning or erasure of fear memories in adults.

There are several alternative mechanisms that may explain the observed lack of conditioned fear responses in ChABC-injected mice. Because degraded PNNs take weeks to turn over after ChABC injection (12), one possibility is that degradation of PNNs could interfere with fear memory consolidation. Although the stability of contextual fear memory in ChABC-injected mice suggests otherwise (Fig. 2C), we specifically tested whether ChABC-injected mice could form a stable, long-term cued fear memory. Mice were injected with ChABC or vehicle (fig. S5) and fear conditioned 24 hours later. Long-term fear memory was ex-

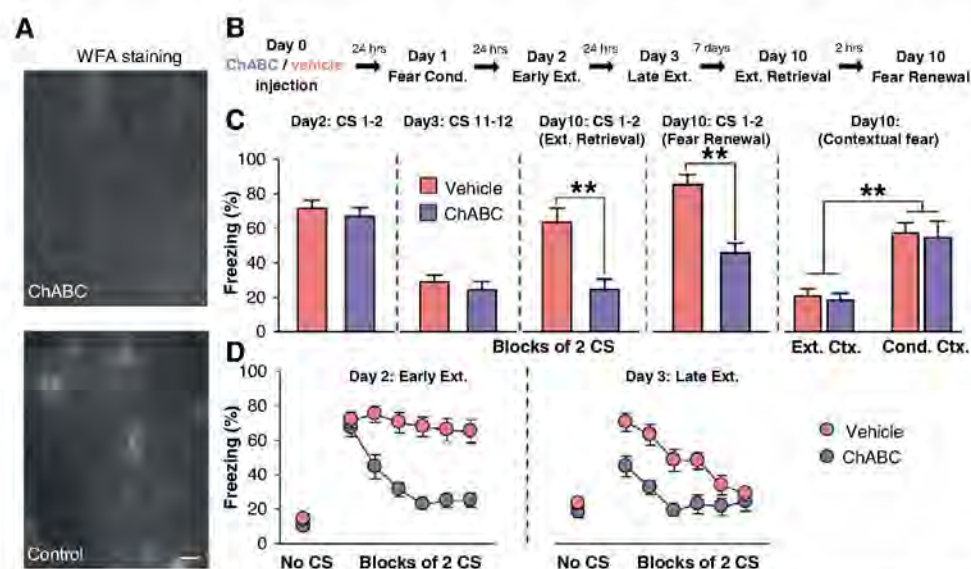


Fig. 2. Degradation of PNNs in adult mice abolishes spontaneous recovery and context-dependent renewal of conditioned fear. (A) ChABC injection eliminates WFA-positive PNNs in adult BLA. Control mice were injected with vehicle (phosphate-buffered saline). (B) Experimental protocol. (C) One week after extinction training, vehicle-injected mice ($n = 10$), but not ChABC-injected mice ($n = 11$), exhibited spontaneous recovery (measured in the extinction context) and renewal (measured in the fear conditioning context) of conditioned fear responses (percent of time spent freezing, extinction retrieval: vehicle: 63.5 ± 8.3 , ChABC: 24.3 ± 6.3 , $P < 0.01$; fear renewal: vehicle: 85.1 ± 6.3 , ChABC: 45.7 ± 5.9 ; $P < 0.01$, two-tailed unpaired t test). ChABC-injected animals exhibited normal contextual fear responses (pre-CS freezing levels measured in the extinction context (Ext. Ctx.) versus conditioning context (Cond. Ctx.; $P < 0.01$ for both groups, two-tailed unpaired t tests). (D) ChABC-injected mice ($n = 13$), but not vehicle-injected mice ($n = 13$), exhibited a rapid reduction in freezing levels as early as the first day of extinction training (Day 2) [two-way ANOVA with repeated measures, (group $\times$ time); group: $F_{(1,24)} = 34.3$, $P < 0.001$; time: $F_{(1,5)} = 12.5$, $P < 0.001$; interaction between group and time: $F_{(5,120)} = 6.9$, $P < 0.001$]. Freezing levels between ChABC- and vehicle-injected mice were significantly different on the second block of extinction (percent of time spent freezing, second block of extinction: vehicle: 75.1 ± 4.7 , ChABC: 44.5 ± 6.9 ; $P < 0.001$, two-tailed paired t test). At the end of the second day of extinction (Day 3), both groups reached similar levels of extinction (percent of time spent freezing, last block of extinction: vehicle: 28.8 ± 4.1 , ChABC: 24.1 ± 5.3 ; $P = 0.51$, two-tailed paired t test). ** $P < 0.01$.

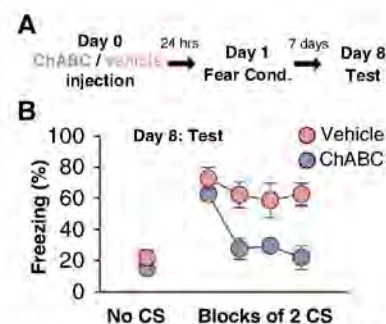


Fig. 3. Degradation of PNNs does not interfere with fear memory consolidation. (A) Experimental protocol for consolidation experiments. (B) Degradation of PNNs did not affect fear memory consolidation as measured 7 days after fear conditioning (percent of time spent freezing, first block: vehicle: 72.8 ± 7.1 , $n = 5$; ChABC: 62.5 ± 3.8 , $n = 5$; $P = 0.78$, two-tailed unpaired t test), yet rapid extinction was induced in ChABC-injected mice exposed to repetitive nonreinforced CSs (one-way ANOVA with repeated measures: vehicle: $F_{(4,3)} = 0.72$, $P = 0.56$; ChABC: $F_{(4,3)} = 18.5$, $P < 0.001$).

aminated 7 days after conditioning (Fig. 3, A and B). Similar to the 24-hour time point, ChABC- and vehicle-injected mice did not differ in terms of their initial freezing levels even 7 days after fear conditioning (Fig. 3B). Still, repetitive CS exposure induced almost instantaneous extinction in ChABC-injected mice, whereas freezing levels remained constant in control animals (Fig. 3B).

A second possible explanation for the lack of spontaneous recovery and renewal could be that removal of PNNs enhanced new learning of inhibitory CS–no US associations during extinction training. That is, ChABC injections may have strengthened the extinction process per se rather than enabled fear memory erasure. Although difficult to test directly, we argued that if removal of PNNs acted by strengthening inhibitory learning during extinction training, the effect of ChABC injection should be independent of whether animals were injected before or after fear conditioning, as long as PNNs were destroyed before extinction training. We therefore compared extinction of conditioned fear memories acquired before or after ChABC injection in the same animals (Fig. 4, A and B; fig. S6). Degradation of PNNs after fear conditioning did not accelerate the time course of subsequent extinction learning (Fig. 4B). In the same animals, however, we observed rapid extinction of a second fear memory acquired in the absence of PNNs (Fig. 4B). Further, repeated retrieval of fear memories acquired before PNN degradation revealed stable freezing levels across several days, thus excluding a possible effect on memory reconsolidation (14, 15) (Fig. 4C). Finally, we examined whether fear memories acquired before ChABC injection could be extinguished normally. Mice fear conditioned 24 hours before ChABC injection exhibited normal extinction and, when tested 1 week later, showed normal levels of spontaneous recovery and context-dependent renewal (Fig. 4, D and E).

Together, these findings demonstrate that loss of PNNs does not strengthen new inhibitory learning during extinction or impair memory reconsolidation, but renders fear memory traces susceptible to unlearning or erasure. Because ChABC injection had only anterograde, but no retrograde effects, we conclude that the state of fear memories acquired in the absence of PNNs fundamentally differs compared with the state of memories acquired in the presence of PNNs. Whereas in the presence of PNNs repeated nonreinforced CS presentations lead to the active inhibition of conditioned fear responses, in the absence of PNNs the same protocol leads to erasure of the fear memory.

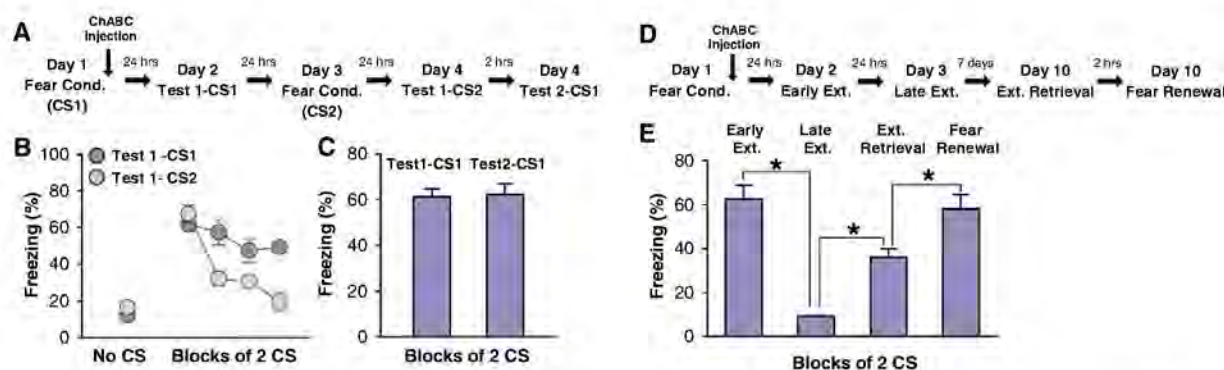
What might be the mechanism by which degradation of PNNs enables fear memory erasure? Fear memories are acquired and stored, at least in part, by learning-induced strengthening of synaptic inputs to the lateral (LA) or basal (BA) nucleus of the amygdala through *N*-methyl-D-aspartate (NMDA) receptor-dependent long-term potentiation (LTP) (1, 16, 17). Thus, one possible mechanism could be that PNNs prevent fear memory erasure by rendering potentiated synapses resistant to LTP reversal, so-called depotentiation (18, 19). However, because removal of PNNs after fear conditioning had no effect on extinction learning, this indicates that PNNs regulate network plasticity during fear conditioning in such a way that fear conditioning leads to the formation of an erasure-prone memory trace. To examine the effect of PNN removal on synaptic plasticity in the amygdala, we compared LTP at thalamo-LA inputs in slices obtained from ChABC- and vehicle-injected animals. LTP of monosynaptic excitatory inputs was completely abolished upon ChABC treatment, and LTP of disynaptic inhibition, which reflects synaptic plasticity at glutamatergic inputs onto local feedforward interneurons, was markedly reduced (fig. S7). Although these results may suggest less potent fear-acquisition mechanisms upon ChABC treatment, they are only correlative

in nature. Different forms of thalamo-LA LTP, or plasticity at other intrinsic or extrinsic synaptic connections and pathways, could be more relevant to the observed behavioral phenotype. Consistent with our observations, however, in vitro removal of PNNs interferes with the induction of LTP and long-term depression in hippocampal slices (20). Underlying mechanisms might also involve changes in local GABA-mediated inhibition. PNNs primarily form around PV-positive GABA-containing interneurons (10), and GABA-mediated inhibition regulates various forms of synaptic plasticity in the BLA (21, 22).

Our study indicates that qualitatively distinct neuronal mechanisms mediate acquisition and extinction of conditioned fear memories during early postnatal development and in adults. In adults, extinction memories coexist with previously acquired fear memories, both of which can be retrieved in a context-dependent manner. By contrast, in young postnatal animals, contextualization of fear memories does not occur—rather, fear memories are overwritten and deleted during extinction. Thus, contextualization of a memory by a second learning episode, a phenomenon that has been proposed to reflect a general principle governing the interaction between different memories (23, 24), appears to be developmentally regulated. Consistent with this notion, the connectivity between brain areas implicated in contextualization of fear and extinction memories, including the amygdala, the prefrontal cortex, and the hippocampus, continues to develop during a protracted period of time, up to several months after birth (25, 26). Functionally, our results may thus imply that during early postnatal development, chances of survival may be optimized by adhering to the most recently learned information. Such a strategy may also support learning from tutors.

Previous results implicate the formation of PNNs around PV-expressing interneurons in critical-period closure in the visual cortex (9, 27).

Fig. 4. Degradation of PNNs does not affect previously acquired fear memories or memory reconsolidation. (A) Experimental protocol. (B) Rapid extinction only occurred for conditioned fear responses that had been acquired after ChABC injection [Fear Cond. (CS2), $n = 6$], but not for those acquired before ChABC injection [Fear Cond. (CS1), $n = 9$, two-way ANOVA, (group $\times$ time); group: $F_{(1,13)} = 9.15$, $P < 0.01$; time: $F_{(1,3)} = 16.87$, $P < 0.001$; interaction between group and time: $F_{(3,39)} = 5.94$, $P < 0.05$]. (C) Degradation of PNNs did not affect fear memory reconsolidation as measured by two consecutive tests separated by 48 hours. (D) Experimental protocol used to examine extinction in animals injected with ChABC after fear conditioning. (E) Normal extinction, spontaneous recovery and renewal in mice injected with ChABC after the acquisition of conditioned fear. Mice were injected with ChABC immediately



after conditioning and submitted to fear extinction. At the end of extinction (Day 3), fear levels were significantly reduced (percent of time spent freezing, early extinction: 62.5 ± 6.3 , late extinction: 9.2 ± 0.3 ; $P < 0.05$, two-tailed paired *t* test). One week later, ChABC-injected mice ($n = 3$) exhibited spontaneous recovery and renewal of conditioned fear responses when tested in the extinction and fear-conditioning context, respectively (percent of time spent freezing, recall: 36 ± 3.9 ; renewal: 58 ± 6.4 ; $P < 0.05$, two-tailed paired *t* test). * $P < 0.05$.

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In the BLA, formation of PNNs marked the end of a developmental period during which fear memories could be erased by extinction and coincided with ability to form contextualized fear and extinction memories. This may suggest a general role for PNNs in mediating developmental changes in information storage in neuronal circuits. However, because degradation of PNNs in adult animals reenables erasure of fear memories by extinction, this demonstrates that the mechanisms underlying extinction-induced fear memory erasure in juveniles are not lost in the adult, but that fear memories are actively protected from erasure by PNNs. Because context-dependent renewal of conditioned fear responses is believed to be an important factor contributing to the relapse of pathological fear in patients undergoing therapy for anxiety disorders (28), our findings may point to novel strategies in preventing the development of extinction-resistant pathological fear and anxiety.

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29. This work was supported by the Austrian Science Fund (FWF), Swiss National Science Foundation, and Novartis Research Foundation. We thank G. Cassasus for electrophysiological characterization of ChABC-injected animals and K. Scheffler for help with programming. We thank R. F. Westbrook and all members of the Lüthi lab for discussions and critical comments on the manuscript. The manuscript was prepared by N.G., P.C., A.L., and C.H. The authors declare that they have no competing financial interest.

Supporting Online Material

www.sciencemag.org/cgi/content/full/325/5945/1258/DC1

Materials and Methods

Figs. S1 to S7

References

27 March 2009; accepted 21 July 2009

10.1126/science.1174146

Activation of the PI3K Pathway in Cancer Through Inhibition of PTEN by Exchange Factor P-REX2a

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PTEN (phosphatase and tensin homolog on chromosome 10) is a tumor suppressor whose cellular regulation remains incompletely understood. We identified phosphatidylinositol 3,4,5-trisphosphate RAC exchanger 2a (P-REX2a) as a PTEN-interacting protein. P-REX2a mRNA was more abundant in human cancer cells and significantly increased in tumors with wild-type PTEN that expressed an activated mutant of *PIK3CA* encoding the p110 subunit of phosphoinositide 3-kinase subunit α (PI3K α). P-REX2a inhibited PTEN lipid phosphatase activity and stimulated the PI3K pathway only in the presence of PTEN. P-REX2a stimulated cell growth and cooperated with a *PIK3CA* mutant to promote growth factor-independent proliferation and transformation. Depletion of P-REX2a reduced amounts of phosphorylated AKT and growth in human cell lines with intact PTEN. Thus, P-REX2a is a component of the PI3K pathway that can antagonize PTEN in cancer cells.

The *PTEN* (phosphatase and tensin homolog on chromosome 10) gene is frequently lost in cancers, and germline *PTEN* mutations are linked to inherited cancer predisposition syndromes (1). The PTEN protein

dephosphorylates phosphatidylinositol 3,4,5-trisphosphate (PIP3), the critical lipid second messenger generated by phosphoinositide 3-kinase (PI3K) upon stimulation of cells by external mitogens (2, 3). Inactivation of PTEN leads to accumulation of PIP3 and, as a consequence, increases activity of the kinase AKT, which promotes cellular survival, cell cycle progression, and growth, thereby contributing to oncogenesis. Studies of human tumors have revealed alterations in multiple components of the PTEN-PI3K-AKT axis, all of which result in increased signaling (4). There is mounting evidence that posttranslational modifications, including oxidation, phosphorylation, and ubiquitinylation, regulate PTEN (5, 6).

To further elucidate cellular regulatory mechanisms, we identified PTEN-interacting proteins by affinity purification. We postulated that a PTEN-mutant cell line would be a rich source for interacting proteins without competing endogenous PTEN and selected DBTRG-05MG, a human glioblastoma cell line, because it grows rapidly in culture and lacks detectable PTEN owing to an in-frame deletion of codons 274 to 342 (7). PTEN-binding proteins were purified from cytoplasmic extracts on an affinity column with PTEN as a glutathione *S*-transferase (GST) fusion protein and sequenced by mass spectrometry (Fig. 1A) (8, 9). Associated proteins included PTEN-binding protein, major vault protein (MVP), and a second protein named P-REX2a (10).

P-REX2a is a guanine nucleotide exchange factor (GEF) for the RAC guanosine triphosphatase (GTPase), which was discovered in a search for proteins with sequence similarity to the leukocyte-specific RAC GEF, P-REX1 (11, 12). P-REX1 GEF activity is critical for RAC-mediated formation of reactive oxygen species in response to PIP3 and signaling by the $\beta\gamma$ heterodimer of heterotrimeric guanine nucleotide-binding protein (G $\beta\gamma$ signaling) in neutrophils (13). P-REX1 expression is increased in metastatic prostate cancers and has been shown to mediate a RAC-dependent metastatic and invasive phenotype in prostate cancer cell lines (14). P-REX2a is a widely expressed paralog of P-REX1 and contains an N-terminal Dbl homology and pleckstrin homology (DHPH) domain (which confers GEF activity), pairs of PDZ and DEP domains, and a C terminus with weak similarity to inositol 4-polyphosphate phosphatase.

To demonstrate an endogenous interaction, we coimmunoprecipitated P-REX2a and PTEN from HEK293 extracts using either a polyclonal

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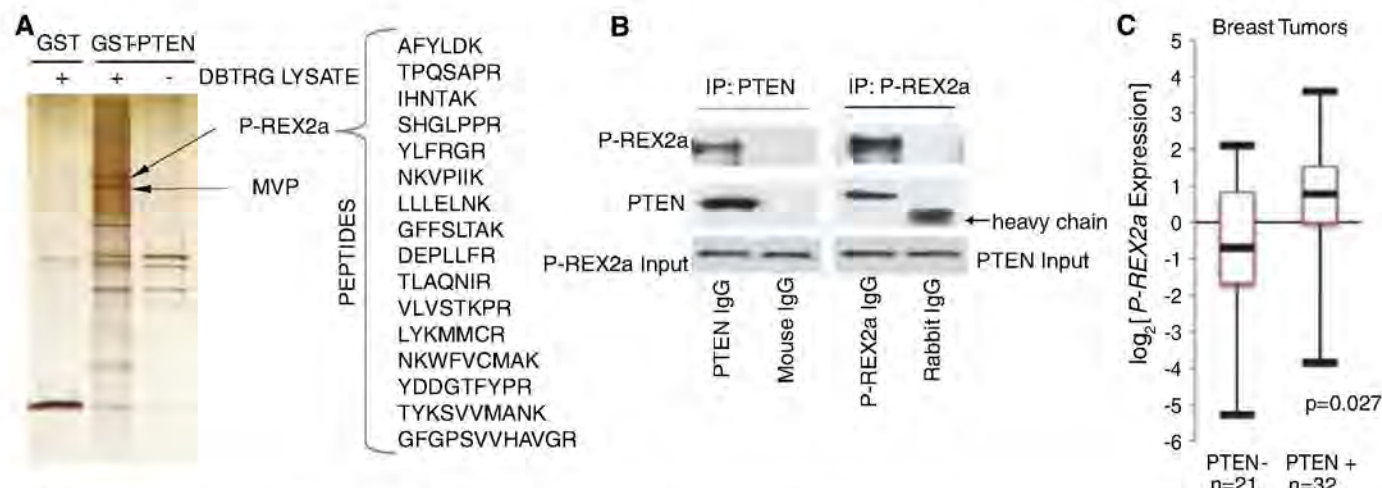


Fig. 1. P-REX2a as a PTEN-binding protein. (A) Silver stain of 1 M salt elutions of affinity-purified PTEN-binding proteins. GST and GST-PTEN columns were incubated with (left and middle) and without (right) DBTRG-05MG cytoplasmic extract, and proteins eluted with high salt were separated and identified by mass spectrometry (9). P-REX2a and MVP are indicated with arrows. Peptide sequences of P-REX2a are shown. (B) Coimmunoprecipitation of endog-

enous PTEN and P-REX2a. Immunoprecipitations were performed (8), and proteins were detected by immunoblotting. (C) Box plot of *P-REX2a* expression in PTEN-positive and -negative breast tumors. Bars above and below represent the maximum and minimum expression, respectively. The box delineates the first to third quartiles of expression, and the central bar represents the median. *P-REX2a* levels are significantly associated with PTEN status ($P = 0.027$) by two-tailed t test.

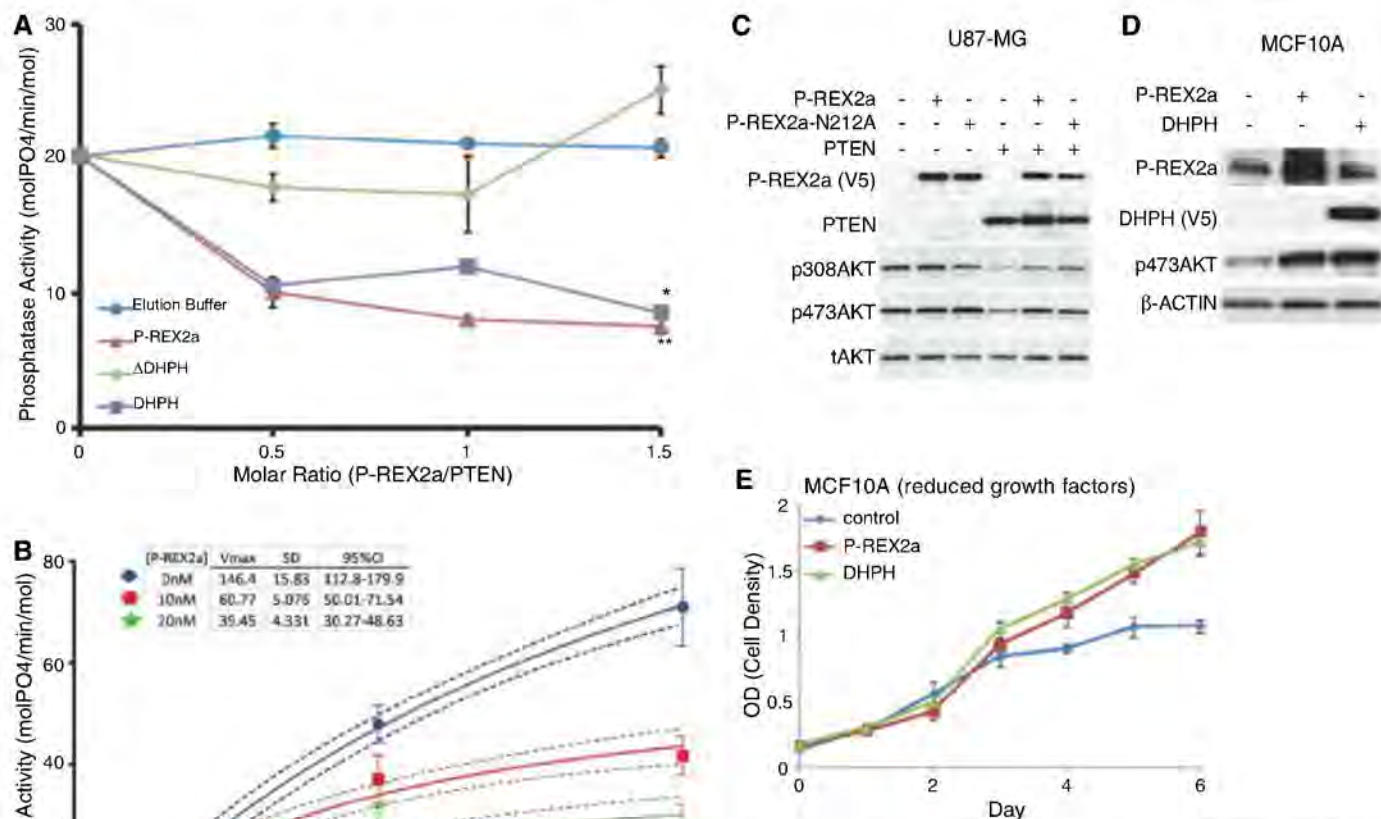


Fig. 2. Inhibition of PTEN phosphatase activity by P-REX2a. (A) Full-length P-REX2a, or a deletion of the DHPH domain (Δ DHPH), or the DHPH domain alone were added in the indicated molar volumes to 40 nM PTEN, purified from HEK293 cells, and phosphatase activity of PTEN was assayed with 20 μ M di-C₈-PIP₃. This is a representative experiment; error bars indicate standard deviation ($n = 3$), $**P < 0.005$, $*P < 0.05$ by ANOVA. (B) P-REX2a (10 nM or 20 nM) was added to the reactions, and the phosphate released was measured over a range of di-C₈-PIP₃ by using 40 nM PTEN. Regression line (solid) to Michaelis-Menten kinetics is shown along with 95% CI (dotted lines). V_{max} values are shown in the table along with standard deviation and 95% CI. A representative experiment is shown, and error bars represent standard deviation ($n = 3$). (C) Effect of P-REX2a and a GEF dead point mutant of P-REX2a, N212A, on phosphorylation of AKT in the presence and absence of PTEN. (D) Effect of expression of P-REX2a alone in MCF10A cells on abundance of p473AKT. The phosphorylation status of T308AKT was not detectable under normal growth conditions in MCF10A cells. (E) Effect of P-REX2a and DHPH on proliferation of MCF10A cells grown in reduced growth factors (0.1% serum). OD, optical density.

antibody to P-REX2a or a monoclonal antibody to PTEN (Fig. 1B). We used Flag-tagged PTEN truncation mutants coexpressed with V5-tagged P-REX2a in HEK293 cells to map the binding domain of PTEN. Deletion of the tail and PDZ-binding domain of PTEN (PTEN Δ Tail, amino acids 1 to 353) disrupted binding to P-REX2a (fig. S1). On the other hand, the C2 and tail domains alone (C2 Δ PDZ) were able to bind to P-REX2a, which indicated that the tail of PTEN, not including the PDZ-binding domain, is required for binding to P-REX2a. In *in vitro* experiments, PTEN bound the DHPH domain, an interaction that was mediated by the PH domain (fig. S2). When overexpressed, PTEN and P-REX2a also displayed subcellular colocalization both diffusely throughout the cytoplasm and at peripheral foci in U87 cells (fig. S3). In stimulation experiments, only those cells exposed to either PDGF or insulin after starvation displayed peripheral foci of colocalization. Starved cells and epidermal growth factor (EGF)-stimulated cells retained diffuse cytoplasmic localization only (fig. S4).

The *P-REX2a* gene is located on chromosome 8q13, a region of frequent amplification in breast, prostate, and colorectal cancers (15–17), which has also been linked to aggressive cancer phenotypes

and metastatic progression (18, 19). We investigated *P-REX2a* expression by quantitative reverse transcription polymerase chain reaction (qRT-PCR) in a breast tumor data set thoroughly annotated for PI3K pathway alterations. *P-REX2a* showed a significant two-tailed association with PTEN status ($P = 0.027$), and the median *P-REX2a* expression was three times that in tumors that retained PTEN as in those that did not (Fig. 1C). Additionally, gene expression data sets from other cancer databases demonstrate increased expression of *P-REX2a* in various tumors, including breast and prostate, compared with that in normal tissues (fig. S5). Mutations in *P-REX2a* were not found in a breast tumor mutation survey (20); however, our analysis of publicly available databases yielded numerous somatic mutations in *P-REX2a* in other tumors, including those of the colon, pancreas, and lung, which make it one of the most commonly mutated GEFs in cancer (fig. S6). We thus suspected that P-REX2a might be a PTEN-regulating factor that is co-opted in tumors to stimulate PI3K signaling.

Affinity-purified full-length P-REX2a (hereafter referred to as P-REX2a) protein (fig. S7) decreased PTEN lipid phosphatase activity at various molar ratios of P-REX2a to PTEN (Fig. 2A) with a sol-

uble lipid substrate di-C₈-PIP₃. At equimolar quantities of P-REX2a, PTEN's phosphatase activity was decreased by $61.6 \pm 2.2\%$ (SD). A decrease of $43.2 \pm 2.8\%$ in phosphatase activity was recorded with the DHPH domain of P-REX2a (hereafter DHPH). Both P-REX2a and DHPH significantly inhibited PTEN activity [$P < 0.005$ for P-REX2a, $P < 0.05$ for DHPH, analysis of variance (ANOVA)]. Conversely, addition of a P-REX2a lacking the DHPH domain (Δ DHPH) showed only modest effects on PTEN activity and was not significantly different than buffer alone ($P > 0.05$, ANOVA).

We measured activity of 40 nM PTEN over a range of substrate (di-C₈-PIP₃) concentrations from 5 to 100 μ M and used nonlinear regression analysis (GraphPad Prism 5.0) to best fit the data to the Michaelis-Menten equation (Fig. 2B). Increasing amounts of P-REX2a resulted in decreases in the maximum phosphatase velocity (V_{max}) of PTEN, indicative of noncompetitive inhibition. We calculated the dissociation constant for inhibitor binding K_i between P-REX2a and PTEN to be 18.97 ± 1.769 nM (SD; 95% confidence interval (CI) = 15.41 to 22.52) (GraphPad Prism 5.0). This high affinity was calculated in the presence of soluble lipid substrate and may be altered at the physiological membrane where PTEN

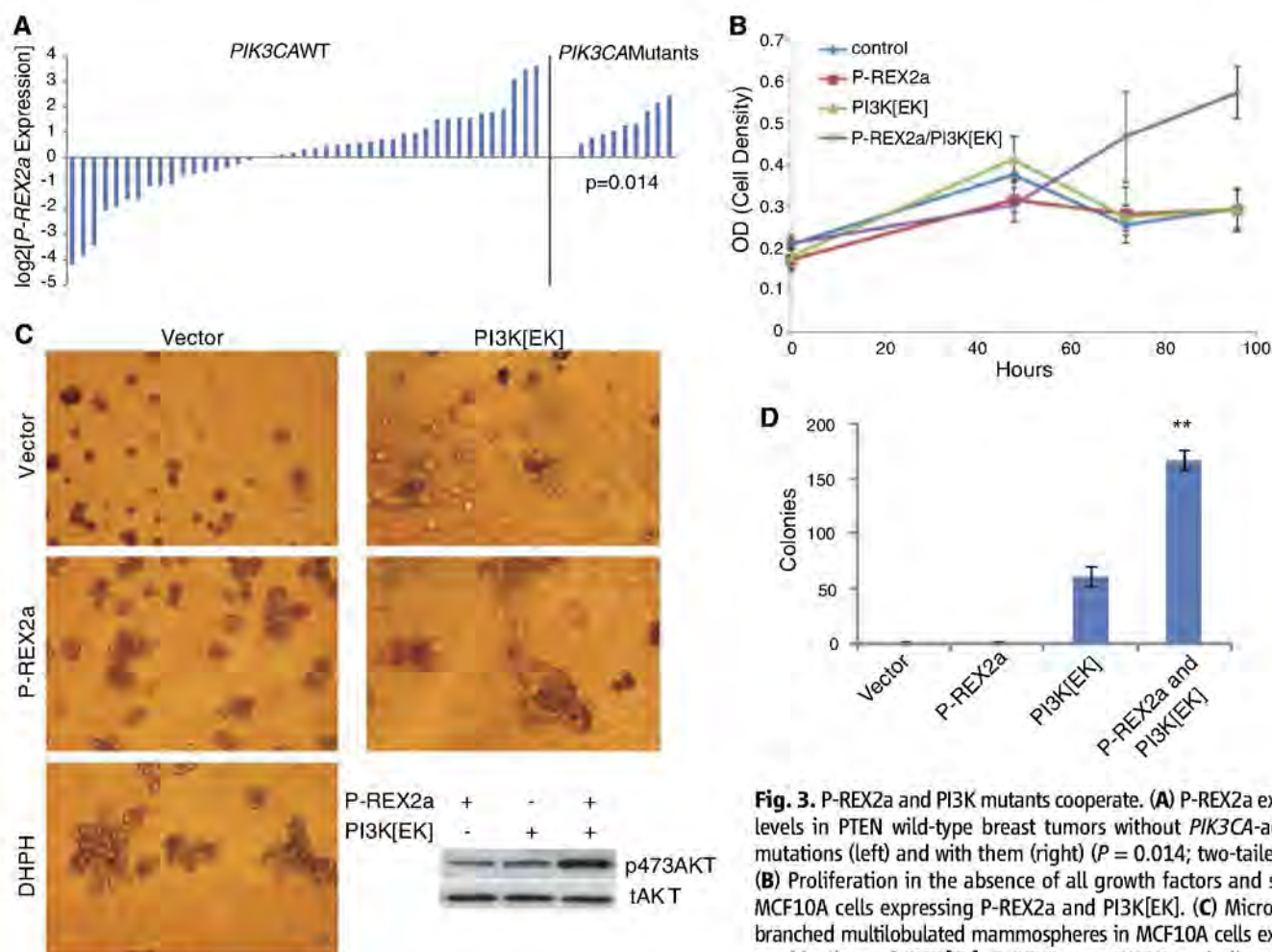


Fig. 3. P-REX2a and PI3K mutants cooperate. (A) P-REX2a expression levels in PTEN wild-type breast tumors without *PIK3CA*-activating mutations (left) and with them (right) ($P = 0.014$; two-tailed *t* test). (B) Proliferation in the absence of all growth factors and serum in MCF10A cells expressing P-REX2a and PI3K[EK]. (C) Microscopy of branched multilobulated mammospheres in MCF10A cells expressing combinations of PI3K[EK], P-REX2a, or DHPH as indicated when grown in matrigel. (Inset) Amount of phospho-473-AKT (p473AKT) and total AKT (tAKT) are shown. All phase-contrast images are displayed at 100 $\times$ magnification. (D) Colony formation by MCF10A cells grown in soft agar. Cells expressing P-REX2a and PI3K[EK] as indicated (** $P < 0.01$; *t* test).

grown in matrigel. (Inset) Amount of phospho-473-AKT (p473AKT) and total AKT (tAKT) are shown. All phase-contrast images are displayed at 100 $\times$ magnification. (D) Colony formation by MCF10A cells grown in soft agar. Cells expressing P-REX2a and PI3K[EK] as indicated (** $P < 0.01$; *t* test).

has been shown to be a highly efficient interfacial phosphatase (21). We also found that coexpression of P-REX2a, but not Δ DHPH, decreased the phosphatase activity of PTEN immunoprecipitated from these cells (fig. S8). Using a point mutant modeled after P-REX1, we further showed that PTEN phosphatase inhibition was independent of the GEF activity of P-REX2a (fig. S9) (22).

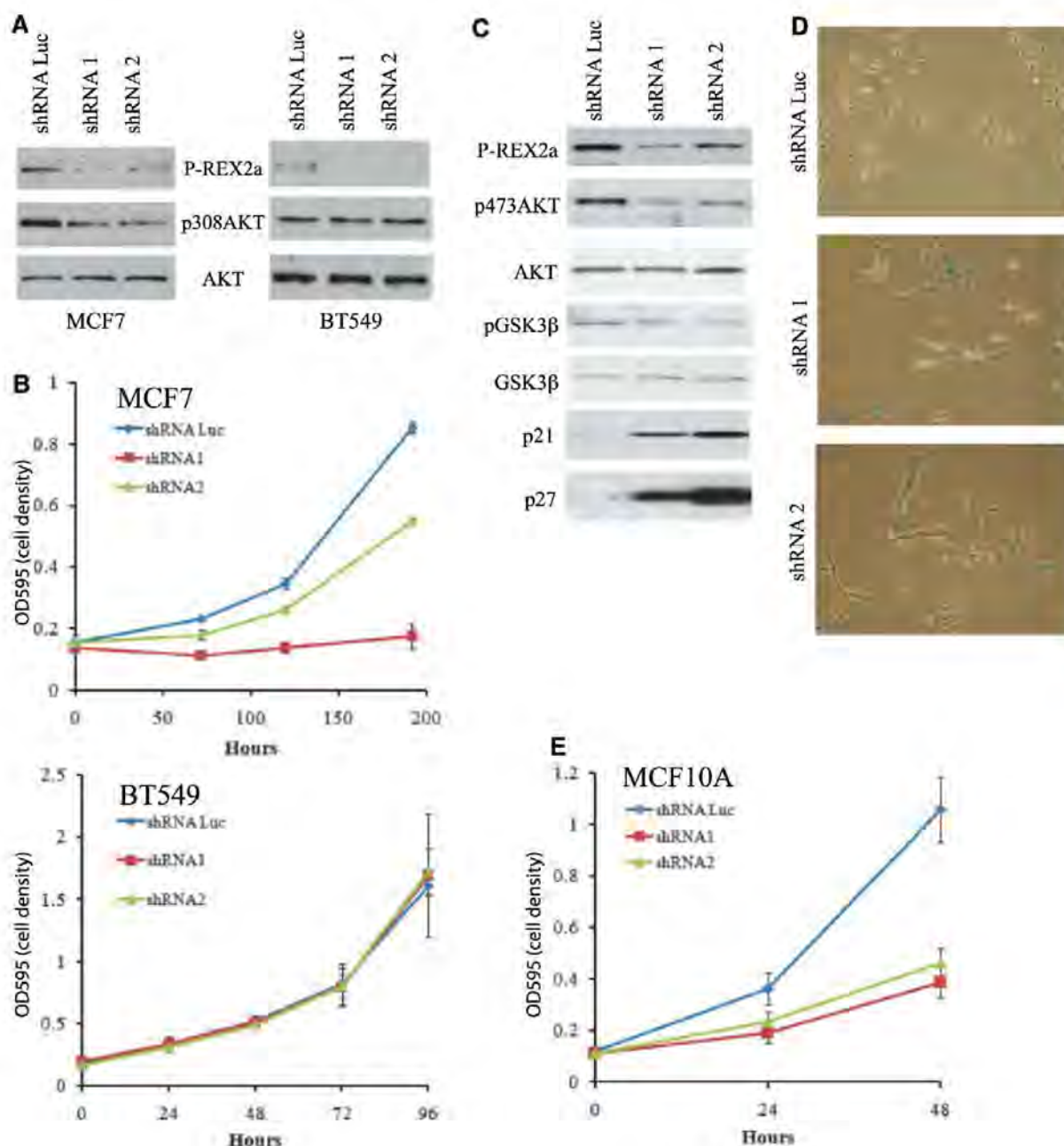
Expression of PTEN in PTEN-deficient U87-MG cells decreased the amount of phosphorylated AKT (pAKT) (Fig. 2C and figs. S10 to S12). Overexpression of P-REX2a had no effect on pAKT alone, but when expressed with PTEN, it restored phosphorylation of AKT at both Ser⁴⁷³ and Thr³⁰⁸ (Fig. 2C). This restoration was independent of P-REX2a's GEF activity but dependent on both the tail (fig. S10) and catalytic activity of PTEN (fig. S11). We observed a similar rescue of

AKT phosphorylation in a second PTEN-deficient glioblastoma cell line (fig. S10). In platelet-derived growth factor (PDGF)-stimulated U87-MG cells (fig. S12), overexpression of PTEN expression alone reduced amounts of phosphorylation of T308AKT, S473AKT, and glycogen synthetase kinase 3 β (GSK3 β). P-REX2a coexpression with PTEN restored levels of phosphorylated AKT and GSK3 β to baseline. Among other agonists investigated, P-REX2a also was able to rescue PTEN suppression of insulin signaling but was ineffectual for those cells stimulated with EGF (fig. S13). Throughout these experiments, we observed that wild-type P-REX2a expression increased levels of simultaneously overexpressed PTEN (Fig. 2C and figs. S11 and S12). This may be a direct result of PTEN inactivation, as previous studies have shown that PTEN stability and activity are inversely correlated (6).

We explored the effect of overexpression of P-REX2a on endogenous wild-type PTEN in an immortalized human mammary cell line, MCF10A. Cells engineered to express either P-REX2a or DHPH exhibited increased amounts of phosphorylation of Ser⁴⁷³ AKT and enhanced proliferation in tissue culture (Fig. 2, D and E). The presence of growth factors was required to observe this difference in proliferation. P-REX2a may not be sufficient to impart growth factor-independent proliferation, because effects of PTEN inhibition would still require PIP3 generation to allow for PI3K activation.

Analysis of P-REX2a mRNA in a cohort of PTEN-expressing breast tumors showed a significant association between increased P-REX2a expression levels and activating mutations in *PIK3CA* ($P = 0.014$, t test) (Fig. 3A). Expression of either P-REX2a or the PI3K α E545K (Glu⁵⁴⁵ replaced

Fig. 4. Diminished phosphorylation of AKT and proliferation after depletion of P-REX2a. Phosphorylation of AKT (A) and proliferation (B) after depletion of P-REX2a in PTEN wild-type MCF7 cells or PTEN-null BT549 cells. OD595, absorbance at 595 nm. (C) Effect of P-REX2a depletion in MCF10A cells on pAKT and pGSK3 β and amounts of the p21 and p27 cell cycle inhibitors. (D) X-gal staining of indicated cell lines. Phase-contrast images displayed at 40 $\times$ magnification. (E) MCF10A proliferation after P-REX2a depletion.



by Lys) constitutively active mutant (PI3K[EK]) alone did not allow growth in the absence of all growth factors, but overexpression of PI3K[EK] and P-REX2a together allowed for growth factor-independent cellular proliferation (Fig. 3B). In three-dimensional matrigel culture, MCF10A cells normally form single-acinar cell clusters; however, expression of either P-REX2a or DHPH resulted in multiacinar epithelial structures, similar to published results of cells expressing activated AKT, ERBB2, or PI3K (Fig. 3C) (23, 24). Expression of PI3K[EK] with P-REX2a led to formation of large, branched, and highly dysmorphic structures and evidence of matrigel invasion. This effect was also observed in soft agar assays in which P-REX2a and PI3K[EK] expression together yielded approximately three times as many transformed colonies as were seen with PI3K[EK] alone ($P < 0.01$, t test) (Fig. 3D). Amounts of phosphorylated AKT increased as well (Fig. 3C, inset).

To test whether endogenous P-REX2a influenced the PI3K pathway in tumor cells, we depleted P-REX2a with short hairpin RNA (shRNA) in a PTEN-wild-type cancer cell line harboring an E545K *PIK3CA* mutation, MCF7, and a PTEN-deficient cell line, BT549. MCF7 cells displayed decreased amounts of pAKT and reduced cell proliferation after introduction of P-REX2a shRNAs, whereas amounts of pAKT and extent of proliferation were not perturbed in BT549 cells (Fig. 4, A and B). Thus, the effects of depletion of P-REX2a on the PI3K pathway and cell proliferation are dependent on the presence of PTEN. Depletion of P-REX2a in MCF10A

cells decreased amounts of pAKT and pGSK3 β (Fig. 4C) and reduced proliferation rate (Fig. 4E). Cells depleted of P-REX2a appeared to be larger, which was confirmed by forward scatter on flow cytometry (fig. S14). Staining of these cells with 5-bromo-4-chloro-3-indolyl β -D-galactopyranoside (X-gal) at pH 6.0, an assay for cell senescence, resulted in marked increase in blue-stained cells in both shRNA knockdown cell lines compared with control cells (Fig. 4D and fig. S14). Increased amounts of the senescence-associated cell cycle inhibitors p21 and p27 were also detected by immunoblot (Fig. 4C). Senescence similarly occurs in human fibroblasts after inhibition of the PI3K pathway by either LY294002 or overexpression of PTEN (25).

Within the PI3K pathway (fig. S15), P-REX2a is poised to amplify PI3K signaling by decreasing catabolism of PIP3. As an activator of the PI3K pathway, P-REX2 may contribute to numerous pathological or physiological processes, such as tumorigenesis, diabetes, and aging. Its place in the pathway as a coordinator of RAC and PTEN makes it an intriguing target for further analysis of signal transduction and therapeutic intervention.

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9. Single-letter abbreviations for the amino acid residues are as follows: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val; W, Trp; and Y, Tyr.
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26. We thank W. Zhang and the Herbert Irving Comprehensive Cancer Center Proteomics Shared Resource, M. Li, W. Gu, V. Seshan, S. Voronov, G. DiPaolo, R. Baer, J. Settleman, K. Kinzler, A. Hall, N. Leslie, C. P. Downes, and the members of the Parsons laboratory for technical assistance or critical reading. B.F. is supported by the NIH Medical Scientist Training Program. Supported by NCI CA097403, the Avon Foundation, Octoberwoman Foundation, and the Adele Meyer Fund.

Supporting Online Material

www.sciencemag.org/cgi/content/full/325/5945/PAGE/DC1
Materials and Methods
Figs. S1 to S15
References

16 March 2009; accepted 15 July 2009
10.1126/science.1173569

Recruitment of Antigen-Specific CD8⁺ T Cells in Response to Infection Is Markedly Efficient

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The magnitude of antigen-specific CD8⁺ T cell responses is not fixed but correlates with the severity of infection. Although by definition T cell response size is the product of both the capacity to recruit naïve T cells (clonal selection) and their subsequent proliferation (clonal expansion), it remains undefined how these two factors regulate antigen-specific T cell responses. We determined the relative contribution of recruitment and expansion by labeling naïve T cells with unique genetic tags and transferring them into mice. Under disparate infection conditions with different pathogens and doses, recruitment of antigen-specific T cells was near constant and close to complete. Thus, naïve T cell recruitment is highly efficient, and the magnitude of antigen-specific CD8⁺ T cell responses is primarily controlled by clonal expansion.

A key feature of adaptive immunity is its ability to recognize a wide range of pathogens by specific antigen receptors expressed on lymphocytes. Because the diversity of antigen receptors is large (1), the frequency of cells specific for any single antigen is extremely

low (less than 1 in 10⁵ cells) (2, 3). This leaves the immune system with the remarkable challenge of selecting those few pathogen-specific cells among the millions of cells that do not recognize a given pathogen at the moment infection occurs. This process of clonal selection is followed by mas-

sive expansion of those selected cells to give rise to sufficient progeny to combat the invading pathogen. Thus, the magnitude of adaptive immune responses is regulated by two processes. First, the number of participating clones is set by the efficiency with which antigen-specific cells are recruited from the naïve repertoire. Second, the burst size of these participating clones, defined as the net sum of all proliferation and cell death, determines the total number of antigen-specific progeny that is generated per recruited cell.

The magnitude of T cell responses generated upon infection is not fixed but is shaped according to the severity of infection (4, 5). Although this correlation is well established, it remains undefined to what extent changes in the magnitude

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of response are the result of an effect on naïve T cell recruitment or clonal burst size. To quantify the number of recruited T cell clones in live infection models, we have labeled individual naïve antigen-specific CD8⁺ T cells with unique DNA sequences (barcodes) (6). Because these barcodes are passed on to all daughter cells, this labeling strategy allowed us to distinguish the progeny of different recruited T cells within the total antigen-specific T cell pool that emerges during infection. Thus, the number of different barcodes detected within an antigen-specific T cell population directly correlates with the number of naïve T cells that have been recruited.

To first investigate the impact of pathogen dose on CD8⁺ T cell recruitment, we labeled OT-I T cell receptor (TCR) transgenic T cells, which are

specific for a peptide fragment of chicken ovalbumin presented by major histocompatibility class I, with a library of barcodes by retroviral transduction. We then transferred 10^3 or 10^4 barcode-labeled green fluorescent protein-positive (GFP⁺) OT-I T cells into C57Bl/6 (B6) mice. One day later, we infected mice with different doses of recombinant *Listeria monocytogenes* bacteria expressing the OVA₂₅₇₋₂₆₄ epitope (LM-OVA) recognized by the OT-I TCR. At the peak of the CD8⁺ T cell response (fig. S1), the total number of barcode-labeled CD8⁺ T cells in spleens was determined. We observed a 10-fold increase in the magnitude of the CD8⁺ T cell response between the lowest and the highest LM-OVA dose (Fig. 1A). In addition, a 10-fold increase in the number of antigen-specific precursors (10^4 OT-I T cells) at a fixed

high-pathogen dose resulted in a further (four-fold) increased T cell response.

To compare the efficiency of T cell recruitment under varying pathogen doses, we determined the number of different barcodes present within the OVA-specific GFP⁺ T cell population. Barcodes were recovered by polymerase chain reaction (PCR) and hybridized to a barcode microarray (6). Analysis of microarray data showed that barcode content obtained from duplicate samples of a single mouse was highly reproducible, indicating that repertoire sampling was reliable (Fig. 1B). Furthermore, use of barcodes between different mice did not overlap. All barcode signals detected were derived from CD8⁺ T cells that had been recruited into the immune response because the signal from non-primed barcode-labeled cells was readily out-competed (fig. S2).

Interestingly, the number of different barcodes recovered from individual mice across the entire range of pathogen doses was highly comparable, with only a 1.5-fold difference in barcode numbers between the lowest and the highest LM-OVA dose (Fig. 1B). This indicates that the profoundly increased magnitude of the CD8⁺ T cell response at higher pathogen dose occurs without a substantial increase in T cell recruitment. Because the magnitude of a T cell response is the product of the number of T cell clones recruited and the burst size of each recruited clone, these data furthermore imply that clonal burst size was changed by sevenfold and thus forms the prime factor regulating T cell responses at varying pathogen doses (Fig. 1C). In contrast, when the available antigen-specific T cell repertoire was raised by infusion of a larger number of T cell precursors, the observed increase in T cell response size (fourfold) was paralleled by an essentially proportional (fivefold) increase in the number of recruited barcodes (Fig. 1, B and C). Notably, near-constant recruitment of T cells at varying pathogen dose was also observed when naïve barcode-labeled OT-I T cells were generated in vivo, through injection of barcode-labeled OT-I thymocytes into the thymus of B6 recipients (fig. S3).

To determine whether near-constant recruitment also applies to other factors known to influence the magnitude of T cell responses, we addressed to what extent recruitment is affected by the duration of infection or by pathogen type and route of infection. Carboxyfluorescein diacetate succinimidyl ester (CFSE) dilution of OT-I T cells responding to LM-OVA infection revealed that ampicillin antibiotic (Amp) treatment limits T cell priming to the first 72 hours postinfection (fig. S4) (7). To study how recruitment is affected by such a reduced duration of infection, we challenged recipients of naïve barcode-labeled OT-I T cells with LM-OVA with or without Amp treatment. Shortening the duration of infection reduced CD8⁺ T cell responses by >10-fold (Fig. 2A). However, the number of naïve T cells recruited was only slightly lowered (~1.5-fold) (Fig. 2, B and C), indicating a change in clonal burst size by >sevenfold. Adoptive transfer of CFSE-labeled OT-I T cells from Amp-treated mice into either LM-OVA or wild-type LM-infected

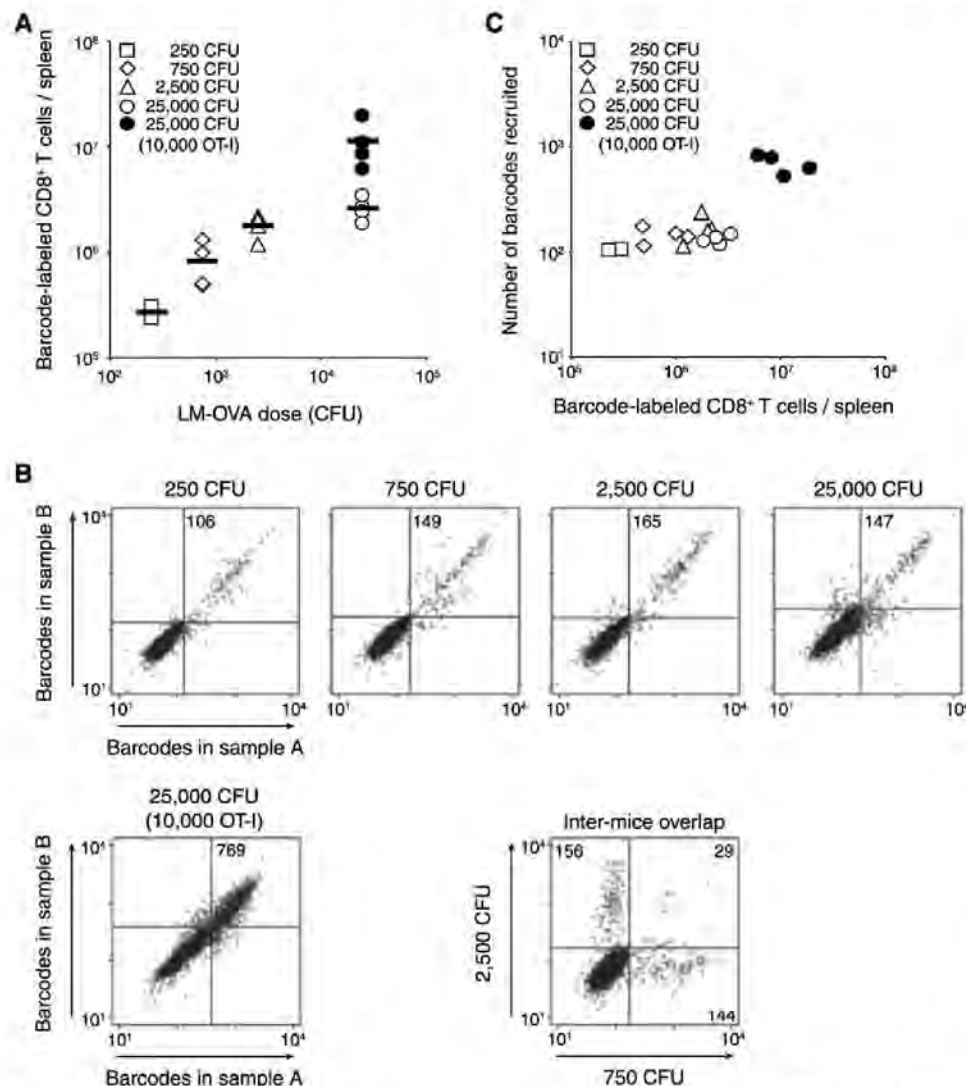


Fig. 1. Effect of pathogen dose and precursor frequency on CD8⁺ T cell recruitment. (A) Magnitude of the barcode-labeled CD8⁺ T cell response. Recipients of 10^3 , or where indicated 10^4 , conA-activated GFP⁺ barcode-labeled OT-I T cells were challenged with the indicated doses of LM-OVA, measured as colony-forming units (CFU). At the peak of the CD8⁺ T cell response (day 8), the number of splenic barcode-labeled CD8⁺ T cells was determined. Symbols represent individual mice; bars represent group mean. (B) Barcode microarray analysis of samples described in (A), showing representative barcode dot plots. In these plots, each dot represents the fluorescent intensity of one barcode of the library. Values indicate the number of barcodes detected above background. Intermice comparison demonstrates that each mouse contains a unique set of barcodes. (C) Comparison of the magnitude of the CD8⁺ T cell response to the number of antigen-specific T cells recruited for all individual mice. Data are representative of three independent experiments.

mice revealed that the enhanced clonal burst size observed upon prolonged infection is primarily due to increased T cell proliferation driven by sustained antigen exposure (fig. S5).

To examine whether near-constant recruitment occurs regardless of pathogen type or route of in-

fection, we challenged recipients of naïve barcode-labeled OT-I T cells by either (i) systemic intravenous LM-OVA infection, (ii) systemic intraperitoneal vaccinia virus-OVA infection, or (iii) local intranasal influenza virus-OVA infection. A strong difference in the magnitude of CD8⁺ T cell responses was

observed, with the response to LM-OVA being >15-fold higher than the response to either vaccinia-OVA or influenza-OVA (Fig. 2D). Despite this major difference in T cell response, the number of recruited naïve T cells in response to these different pathogens was highly comparable (Fig. 2, E and F).

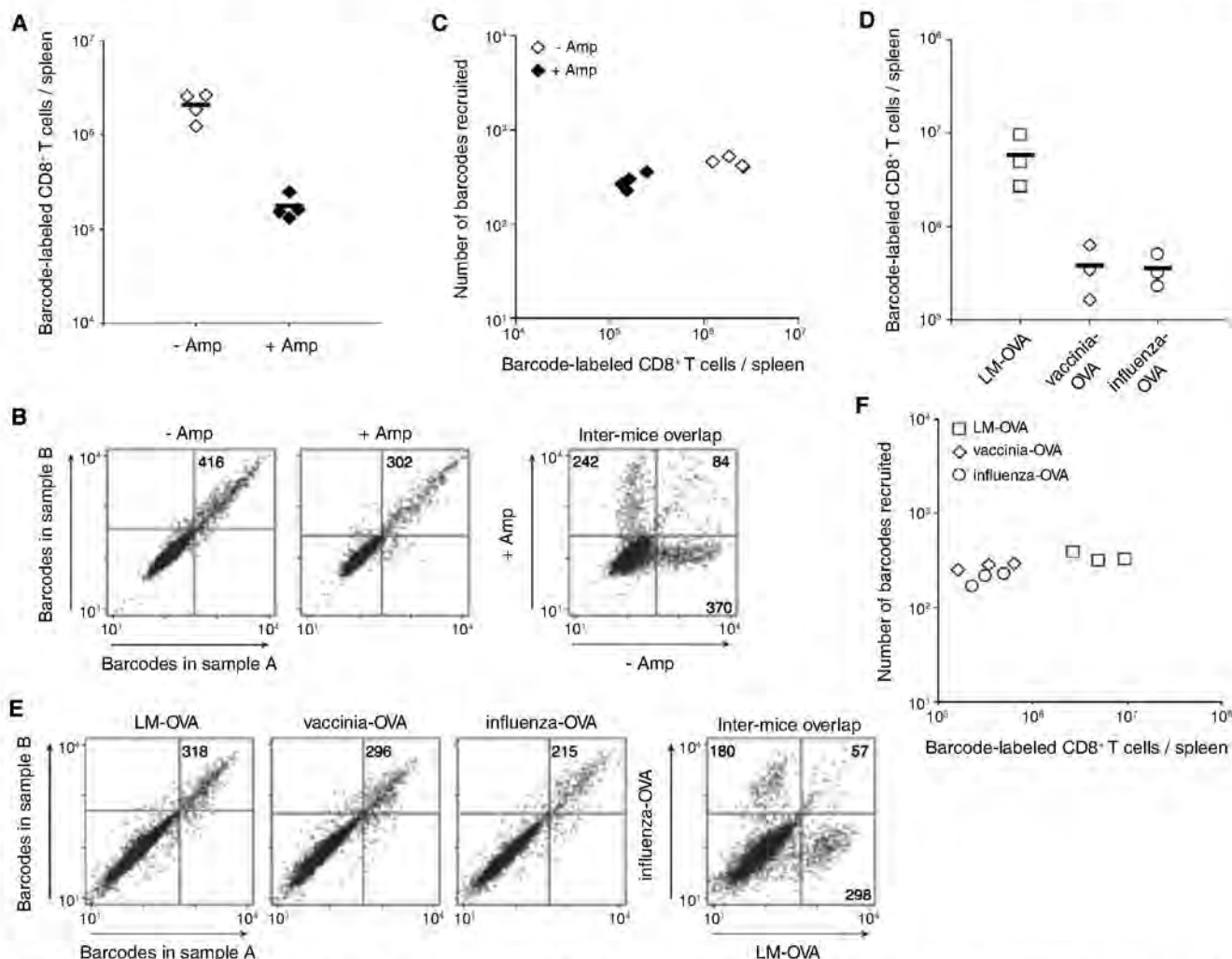


Fig. 2. Effect of duration of infection and pathogen type on CD8⁺ T cell recruitment. (A) Magnitude of the barcode-labeled CD8⁺ T cell response of recipients of $\sim 10^3$ naïve barcode-labeled OT-I T cells that were infected with LM-OVA with or without Amp treatment. (B) Representative barcode dot plots of samples described in (A). (C) Comparison of the magnitude of the CD8⁺ T cell response to the number of antigen-specific T cells recruited for all individual

mice. (D) Magnitude of the barcode-labeled CD8⁺ T cell response of recipients of $\sim 10^3$ naïve barcode-labeled OT-I T cells that were infected with LM-OVA, vaccinia-OVA, or influenza-OVA. (E) Representative barcode dot plots of samples described in (D). (F) Comparison of the magnitude of the CD8⁺ T cell response to the number of antigen-specific T cells recruited for all individual mice. Data are representative of two independent experiments.

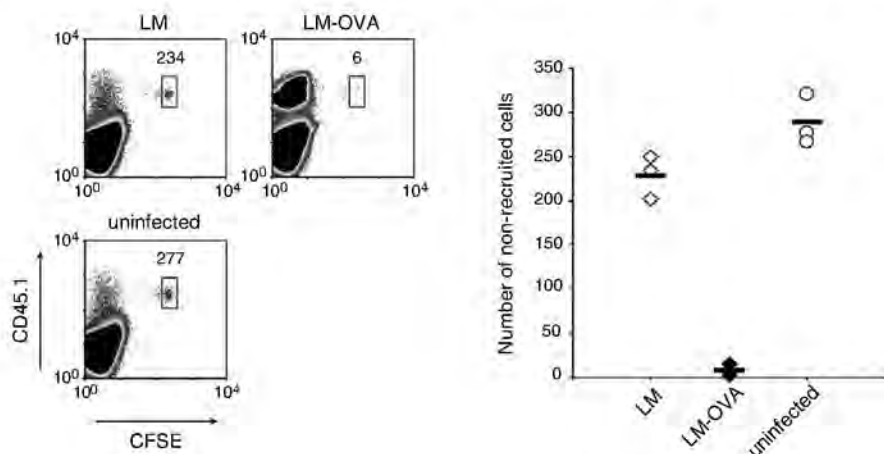


Fig. 3. Antigen-specific CD8⁺ T cell recruitment upon infection is close to complete. CD45.2 recipients of 5×10^3 CFSE-labeled CD45.1⁺ OT-I T cells were infected with wild-type LM, LM-OVA, or left uninfected. At the peak of the CD8⁺ T cell response, total spleen and lymph node CD8⁺ T cells were analyzed by flow cytometry. (Left) Representative flow cytometry plots gated on live CD8⁺ T cells; values indicate the number of nonrecruited (CFSE^{hi}) OT-I T cells. (Right) Cumulative data for each group. Data are representative of six independent experiments.

The above data indicate that naïve T cell recruitment is highly constant but do not reveal its efficiency. To directly enumerate recruitment efficiency, we quantified the number of antigen-specific T cells that do not undergo clonal expansion after infection. To this purpose, CD45.2 recipient mice received 5×10^3 CFSE-labeled congenic CD45.1⁺ OT-I T cells and were challenged with either LM-OVA, wild-type LM, or left uninfected. Within the roughly 2×10^7 CD8⁺ T cells recovered from pooled secondary lymphoid organs of wild-type LM-infected and uninfected mice at day 8 postinfection, we detected on average

228 ± 24 and 288 ± 29 CFSE^{hi} (nonrecruited) OT-I T cells, respectively (Fig. 3). In contrast, in mice infected with LM-OVA only a very low number (8 ± 7) of remaining CFSE^{hi} cells could be detected. This indicates that >95% of the OT-I T cell pool was selected to undergo clonal expansion when the OVA antigen was present and thus recruitment was close to complete.

Although the dissociation constant of the OT-I - K^b-OVA interaction ($K_d = 6 \mu\text{M}$) (8) is representative of the interactions observed for T cells that recognize foreign antigens (0.1 to 20 μM) (9, 10), it was important to establish whether

polyclonal T cell populations expressing a range of TCR affinities follow similar patterns of recruitment. To address this issue, we made use of "Limited" (Ltd) mice that express the OT-I TCR β chain transgene together with a V α 2 TCR α chain minilocus (11). Rearrangement of this minilocus has been shown to result in the formation of a restricted repertoire of CDR3 α variants (~40 different TCRs were described in lymph node CD8⁺ T cells). Consistent with the hypothesis that CD8⁺ T cells of Ltd mice display variable affinities for the OVA antigen, Ltd CD8⁺ T cells showed a range of K^b-OVA tetramer binding (Fig. 4A). Furthermore, similar to what was observed for OT-I T cells (Fig. 1 and fig. S3) and endogenous OVA₂₅₇₋₂₆₄-specific CD8⁺ T cells (fig. S6), an increased magnitude of the Ltd CD8⁺ T cell response was observed at increasing LM-OVA doses, with a sixfold difference between the lowest and the highest dose (Fig. 4B). Nevertheless, recruitment of Ltd CD8⁺ T cells was only minimally altered (<1.5-fold; Fig. 4, C and D). Importantly, at both low and high antigen doses, the vast majority of recruited Ltd cells was characterized by two TCR α protein sequences (94.3% and 95.5% of sequences, respectively) (Fig. 4E). Furthermore, the relative proportion of these two TCR α protein sequences in the responding T cell population was identical between low and high dose-challenged mice. Notably, these two TCRs, which have near-identical affinity for the OVA antigen (fig. S7), were both encoded by a high number of different CDR3 α DNA sequences (table S1), indicating that these Ltd responses are highly polyclonal at the DNA level and selected for "fitness" at the protein level.

Under highly disparate conditions of infection, naïve CD8⁺ T cell recruitment is near constant and close to complete. This indicates that control of the magnitude of antigen-specific CD8⁺ T cell responses primarily occurs through regulation of clonal burst size. The observed efficiency of recruitment also indicates that the process through which naïve T cells scan antigen-presenting dendritic cells (DCs) must be sufficiently robust to allow the vast majority of antigen-specific T cells to encounter a DC carrying cognate antigen within the first 72 hours of infection. Assuming that naïve antigen-specific CD8⁺ T cells are present at a frequency of ~1:100,000 within a CD8⁺ T cell pool of $\sim 20 \times 10^6$ cells, it would require around 59×10^6 T-DC interactions to achieve 95% recruitment, a number that is largely independent of variations in precursor frequency within the physiological range (12). It has been estimated that DCs are able to interact with at least 500 different T cells/hour (13, 14); thus, a pool of <2000 antigen-presenting DCs could suffice to achieve this near-complete recruitment.

How can participation of high affinity clones be highly efficient even at low pathogen doses, with little evidence for participation of additional clones at greatly increased pathogen levels? Previous studies of the CD4⁺ T cell response to injected soluble antigens have shown that a high proportion of the TCR diversity found in the naïve

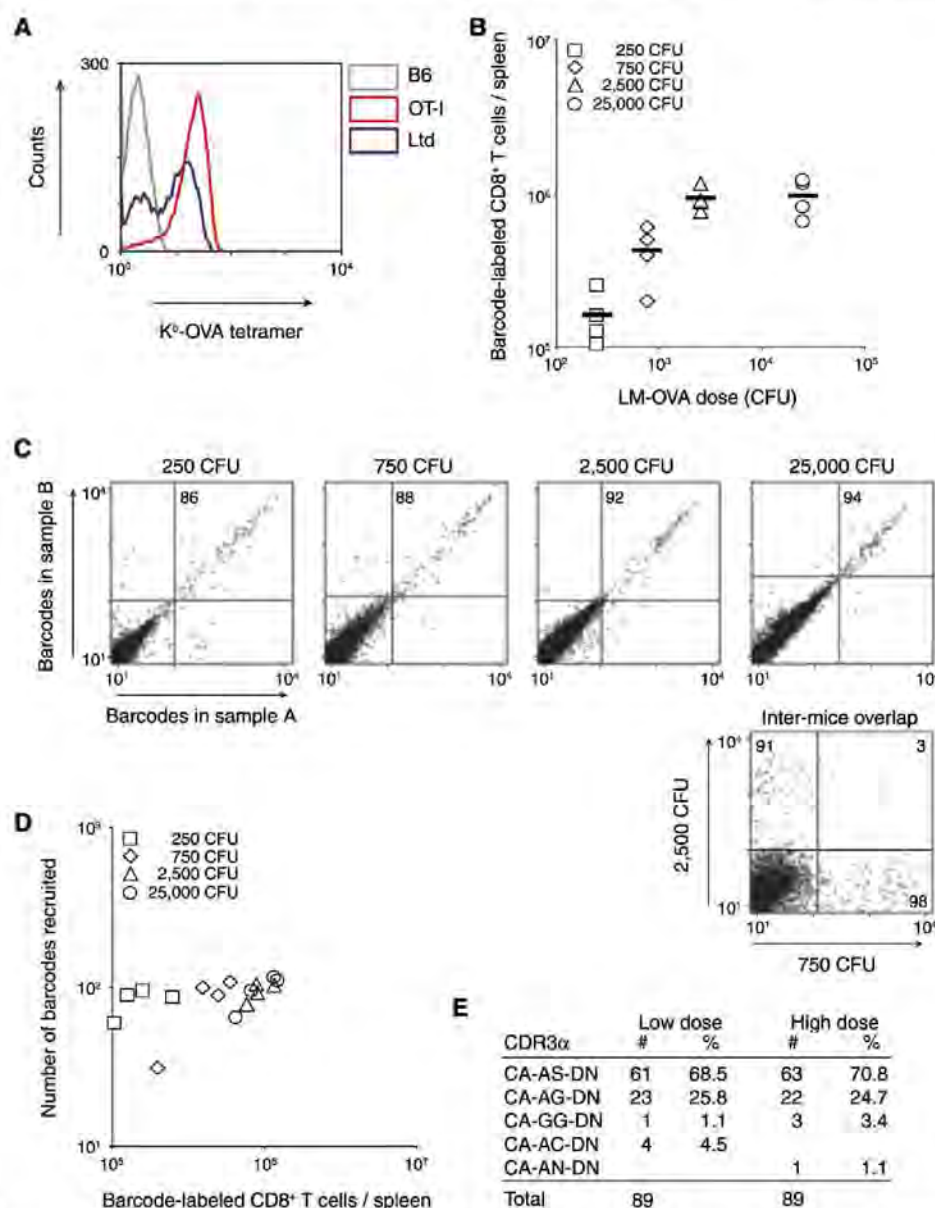


Fig. 4. Efficiency of T cell recruitment of polyclonal T cell populations. (A) K^b-OVA tetramer binding of B6, OT-I, and Ltd CD8⁺ T cells. Representative histogram gated on live CD8⁺ T cells is shown (two mice per group). (B) Magnitude of the barcode-labeled CD8⁺ T cell response of recipients of 10^3 barcode-labeled Ltd CD8⁺ T cells that were challenged with indicated LM-OVA doses. (C) Representative barcode dot plots of samples described in (B). (D) Comparison of the magnitude of the CD8⁺ T cell response to the number of antigen-specific T cells recruited for all individual mice. (E) CDR3 α TCR sequences of Ltd CD8⁺ T cells responding to infection. Data represent pooled sequences of two low (250 CFU) and two high (25,000 CFU) dose infected mice. Note that the dominance of the CDR3 α amino acid sequences CA-AS-DN and CA-AG-DN (which compose 35% and 19% of the naïve Ltd repertoire, respectively) (11) increases to a combined 95% upon antigen-driven selection.

repertoire was also preserved during the early stages of antigen-induced proliferation (2, 15). Notably, one of these studies showed that, whereas early during the response TCR diversity was still highly reflective of the naïve repertoire, at the peak of the immune response TCR diversity was skewed toward higher-affinity clones (15). In line with this, recent data have shown that lower-affinity interactions do result in T cell activation in vivo, but these responses undergo premature contraction (16). Together with our data, these studies support a model in which the immune system maximizes its potential to react toward invading pathogens by allowing a near-complete recruitment of high-affinity T cells, independent of the conditions of infection (fig. S8). Although this may lead to concomitant activation of lower-affinity cells, the abortive expansion of these clones

forms a filter against their further participation (15, 16).

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Supporting Online Material

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27 April 2009; accepted 20 July 2009

10.1126/science.1175455

Differential Sensitivity to Human Communication in Dogs, Wolves, and Human Infants

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Ten-month-old infants persistently search for a hidden object at its initial hiding place even after observing it being hidden at another location. Recent evidence suggests that communicative cues from the experimenter contribute to the emergence of this perseverative search error. We replicated these results with dogs (*Canis familiaris*), who also commit more search errors in ostensive-communicative (in 75% of the total trials) than in noncommunicative (39%) or nonsocial (17%) hiding contexts. However, comparative investigations suggest that communicative signals serve different functions for dogs and infants, whereas human-reared wolves (*Canis lupus*) do not show doglike context-dependent differences of search errors. We propose that shared sensitivity to human communicative signals stems from convergent social evolution of the *Homo* and the *Canis* genera.

Convergent findings indicate that human infants pay special attention to various nonverbal communicative signals directed at them [such as eye contact, gaze-shifts, and pointing (1–5)]. This skill may provide the basis for preverbal infants' early emerging competence to engage in triadic communicative interactions with others (6–8). Recent evidence suggests that signals conveying manifestation of intention to communicate induce a learning attitude in infants, which enables them to acquire knowledge from observation of adults' demonstrations (9). In brief, infants are biased to assume that the information manifested by the adult can be generalized to other situations. We have shown (10) that this bias can account for young infants' perseverative search error (11) in the A-not-B object search task. In our study with 10-month-olds (10),

participants observed a human demonstrator repeatedly hiding (and then allowing the infant to retrieve) an object at one of two potential hiding locations: four times at location A and then 3 times at location B, either in a communicative context or without any social cues. In the communicative context, we replicated the standard finding of a strong tendency to search (erroneously) for the object at its previous hiding location (A) during the B trials. This perseverative bias has been substantially reduced, however, when no communicative cues accompanied the object-hiding actions. The robust association between the ostensive-communicative context of the hiding actions and the perseverative search error supports the "natural pedagogy" hypothesis (9), according to which the perseverative error is in large part due to a pragmatic misinterpretation of the experimenter's hiding actions as constituting a communicative teaching demonstration rather than being just a hide-and-search interactive game.

Humans are not the only species that show special sensitivity to human ostensive-referential signals. Recent results indicate a functionally sim-

ilar sensitivity and preference in dogs for certain nonverbal cues of human ostensive and referential communication (12). Unlike great apes (13), dogs exhibit some understanding of human referential intentions expressed in communicative gestures, such as pointing, as shown by their success in solving the so-called object choice tasks (14, 15).

To investigate the functional nature of dogs' sensitivity to ostensive-referential cues in a comparative manner, we used the A-not-B object search paradigm that had been used to demonstrate the influence of communicative cues on human infants' perseverative search errors (10). In the first experiment, we tested whether communicative signals would have a notable effect on dogs' tendency to persevere in a search task. Three groups of adult dogs (12 in each) participated in a task involving searching for an object that they saw being hidden behind one of two identical screens. In the first phase, the dog was allowed to fetch a toy repeatedly from behind the screen where it was hidden (four A trials). In the test phase, the experimenter repeatedly hid the toy object behind the alternative B screen (three B trials). Each dog participated in one of the following three conditions:

In the social-communicative (SocCom) condition, the hider attracted the dog's attention by ostensive addressing signals ("[dog's name] + Watch!"). Then she picked a rubber ball from the floor while establishing eye contact and addressing the dog ("Watch!") and walked to screen A with the toy in her hand being constantly visible to the dog. As she placed the ball behind screen A, she displayed gaze shifts looking back and forth between the hiding location and the dog (A trials). During the B trials, she passed behind screen A with the ball visibly held in her hand and moved on to screen B, placing the object behind it. After showing her empty hands to the dog, the subject was allowed to choose which screen to go behind to retrieve the toy.

In the noncommunicative (NonCom) condition, the experimenter performed the same object-hiding manipulations as in the SocCom condition with her back turned toward the dog. Thus, neither

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eye contact nor facial cues were displayed while the experimenter held the object in her hand clearly visible to the subject. In addition, she did not talk to the dog but attracted the dogs' attention by clapping her hands and making a conspicuous noise with the toy.

In the nonsocial (NonSoc) condition, the experimenter remained still next to the dog while another experimenter, who was invisible to the dog, made the ball move behind the screens by pulling a transparent string (invisible to the dog) to which it was attached. No communicative signals were displayed toward the dogs.

Dogs' search responses were categorized as correct (selecting the baited location scored 1), ambiguous (score of 0.5) or incorrect (selecting the nonbaited location scored 0) (16). Dogs fetched the object reliably during the A trials in all three conditions (mean percentage of correct choices were 94% in SocCom and 98% in NonCom and NonSoc groups). However, during the B trials we found striking context-dependent differences in the number of dogs committing the A-not-B error (table S1), and a one-way analysis of variance (ANOVA) on the response scores also showed highly significant differences ($F_{2,33} = 10.436$, $P < 0.001$). Post-hoc pairwise comparisons (Tukey–Kramer test) revealed that dogs in the SocCom condition searched at the baited screen (B) less often than those in the NonCom condition ($P < 0.05$) or in the NonSoc condition ($P < 0.001$). In addition, dogs in the SocCom condition displayed a search bias toward the empty (A) screen because they performed well below the success rate expected by random search ($t_{11} = 3.576$, $P = 0.004$). In contrast, dogs in the NonSoc condition were significantly more successful than chance during the B trials ($t_{11} = 3.867$, $P = 0.003$) (Fig. 1).

These results clearly indicate that, similarly to human infants (10), the communicative context induced in dogs a tendency to perseveratively (and erroneously) search for a hidden object at a previously repeatedly baited location (A) even when they observed the object being hidden at a different location (B). This error, however, has been eliminated when the hiding events were not accompanied by communicative signals. Thus, contrary to previous accounts (17, 18), the perseverative search tendency found in both species cannot be explained as stemming from an inability to locate hidden objects or to inhibit a search response at a previously rewarded location (A). Moreover, if the social-communicative signals simply had a distracting effect, one would expect random search and not an explicit bias to the empty location (A), which we found in the SocCom condition. Therefore, we propose that search error in dogs and infants may be indicative of their shared social competence that involves preparedness for learning from humans through communication.

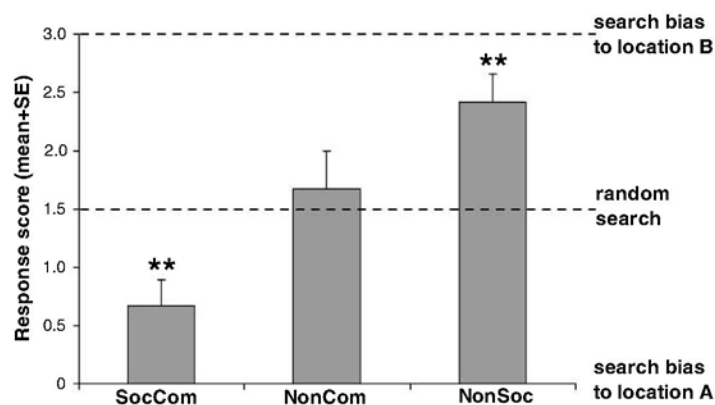
One intriguing question is whether dogs' sensitivity to human communicative signals is the evolutionary consequence of domestication. It is

increasingly assumed that, during their evolution in an anthropogenic environment (and paralleled by the divergence from the wolf), dogs have become selected to display increased sociality (19, 20), cooperability (21), and communicability (22, 23). This preparedness enables the dog to become sensitized to human communicative cues (12) if the individual is properly socialized to humans (24).

This account predicts that only dogs but not human-reared wolves would respond differentially to communicative versus nonsocial hiding contexts in a search task. We tested this prediction in experiment 2, in which we compared the performance of a different group of naïve pet dogs ($n = 12$) to that of 10 extensively socialized, hand-reared wolves [for more details on socialization of wolves, see (19–21)] in the SocCom and NonSoc conditions. The procedure was similar to that which was used in experiment 1 except that the bait was a piece of food in a small plastic cup, and dogs and wolves participated in both the communicative and the nonsocial hiding conditions (within-subject design) (16).

Dogs and wolves selected the baited location reliably during the A trials in both conditions (mean percentage of correct choices were 95% and 88% for wolves and 94% and 92% for dogs in the SocCom and NonSoc conditions, respectively). However, the performance during the B trials differed markedly between species and contexts (Fig. 2 and table S2). A two-way ANOVA on the response scores for hiding context and species as factors revealed more correct responses in the NonSoc than in the SocCom condition ($F_{1,20} = 15.003$, $P = 0.001$), more correct responses by the wolves than by the dogs ($F_{1,20} = 4.675$, $P = 0.043$), and a significant interaction between these factors ($F_{1,20} = 13.027$, $P = 0.002$). This interaction was due to the fact that, similar to experiment 1, dogs selected the baited location on B trials less frequently in the SocCom condition ($t_{11} = 5.043$, $P < 0.001$), whereas no such effect was found in wolves ($t_9 = 0.208$, $P = 0.840$).

Fig. 1. Scores of correct responses (mean $\pm$ SE) in the B trials as a function of the hiding context. The dogs ($n = 12$ for each condition) received four A trials followed by three B trials. The SocCom condition was that the human experimenter repeatedly hid a toy object behind screen A and then behind screen B using ostensive-communicative signals. The NonCom condition was that the experimenter performed the same object-hiding manipulations as in the SocCom but without ostensive-communicative signals. The NonSoc condition was that the experimenter remained still next to the dog while the object moved behind the screens without any perceivable human manipulation. ****** $P < 0.01$, one-sample t test, in comparison with the success rate expected by random search (0.5 times three B trials).



The robust A-not-B error in the communicative hiding context in dogs, which was absent in extensively socialized wolves, represents a striking interspecies difference, which could be best explained by assuming that selective processes in the course of domestication of dogs led to sensitivity to human ostensive and referential signals (21, 25). However, the fact that dogs, like human infants, commit the perseverative search error when (and only when) the repeated hiding events are presented in a communicative context does not necessarily imply that this effect is mediated by the activation of the same type of interpretive bias that the ostensive cues were hypothesized to trigger in human infants (9, 10). Evidence suggests that dogs' response to human communication is primarily driven by a motivation to satisfy ostensively cued human imperatives even when the human's action demonstration conveys an inefficient or mistaken solution to goal approach (26), food choice (27), or object choice (28). These findings, along with the results presented in experiments 1 and 2, raise the question of whether human ostensive and referential signals serve the same communicative functions in dogs that they do in human infants. If human communication is functionally interpreted as imperatives by dogs, it might be tied to the situational context, whereas infants, whose primary motivation is to learn from ostensive demonstrations, would attempt to generalize the communicative content to new situations.

Because one of the crucial components of the A-not-B task is the identity of the person they interact with, in experiment 3 we investigated how dogs and 10-month-old human infants react if, after the A trials, the identity of the hiding person is changed and a new experimenter continues the hiding during the B trials in the SocCom condition. If the ostensive hiding action is interpreted as an imperative order associated with a specific "instructor," we could expect the perseverative search bias to diminish during the B trials, which would represent a different imperative to act on because it is given by a different

person. In contrast, if the ostensive hiding action is interpreted (or misinterpreted) as conveying some generalizable information about the type of object hidden or the function of the hiding location (9) that is not related to the identity of the particular demonstrator, switching the experimenter should not reduce the tendency to commit A-not-B error.

For naïve infants ($n = 12$), we applied the same procedure used by Topál *et al.* (10) in the ostensive-communicative hiding context, and the procedure for a group of naïve dogs ($n = 12$) was similar to that which was used in the SocCom condition of experiment 1. However, after the experimenter had repeatedly hidden the toy in the A trials, she left and another familiar person continued to demonstrate the hiding actions during the B trials (16). During the A trials, dogs fetched the object reliably from behind screen A (mean percentage of correct choices was 98%), showing a performance similar to that found in the SocCom condition of experiment 1 (94%). Infants also searched for the toy correctly in the majority of A trials (82%), replicating the success rate (88%) reported by Topál *et al.* (10) in the same ostensive-communicative hiding context. However, infants and dogs responded to the new experimenter in the B trials differentially (Fig. 3 and table S3). Infants displayed a perseverative search bias to reach toward location A, and their success rate was significantly below chance level ($t_{11} = 2.932$, $P = 0.014$). In contrast, dogs did not show a significant search bias toward the empty A location ($t_{11} = 0.103$, $P = 0.920$), suggesting

that they did not generalize to the new situation in the B trials what they had learned during the A trials.

These results show the differential influence of changing a basic stimulus parameter (the identity of experimenter) on dogs' and human infants' tendency to commit the perseverative search error. The finding that, as compared with the SocCom condition in Experiment 1, dogs did not perseverate after switching the experimenter is consistent with the hypothesis that dogs anchor communication to the specific situation and especially to the specific communicator who is ostensively addressing it to them. At the same time, and unlike in the NonSoc condition of Experiment 1, dogs were not always successful in finding the object hidden by the new experimenter. Their random search pattern indicates that the unchanged aspects of the situation (such as same object, same room, and same screen) carried sufficient cues of the previous context to confuse them and suggests that it is the overall similarity of the test situation to the training situation that determines whether dogs extend the scope of the learned imperative to the new context. Crucially, the human who ostensively communicates toward the dog forms an indispensable element of the context. For infants, however, it does not seem to matter who performs the ostensive hiding demonstration, and they readily generalize their erroneously learned object-finding action to the new-person context. This result provides further validation for the natural pedagogy hypothesis (9) as an explanatory

framework of the Piagetian A-not-B error in infants (10).

In summary, these results show an apparent behavioral analogy between human infants and dogs. In both species, one of the most important causal factors leading to perseverative search errors is the communicative ostensive-referential context. The seemingly mistaken response, called A-not-B error, is not (or at least not only) due to insufficient attentional and motor functioning but paradoxically may be indicative of sophisticated social competence in both dogs and human children. However, the precise function of the cognitive-interpretive mechanisms elicited by communication differs between dogs and humans. For infants, ostensive and referential communicative signals serve a primarily epistemic function by indicating an opportunity to acquire culturally shared knowledge about referent kinds (9). Dogs' sensitivity to these signals is parasitic on human communication by exploiting them for a different function: to give orders to perform some specific action at a referentially indicated particular location in the presence of (and for) a specific person presenting the imperative.

The lack of a similar sensitivity to human ostensive-communicative signals in extensively socialized wolves supports the view that this is an evolutionary novel skill in the *Canis* genus, providing a typical case for convergent social evolution (as the consequence of domestication) between man and dog. In addition to human-ape comparisons, the study of the behavioral convergence between dogs and humans (29) offers a comprehensive framework for understanding the evolutionary emergence of human social behavior.

Fig. 2. Comparison of dogs and wolves in the A-not-B task. Scores of correct responses (mean + SE) in the B trials as a function of the hiding context. ### indicates $P < 0.0001$ in a paired t test.

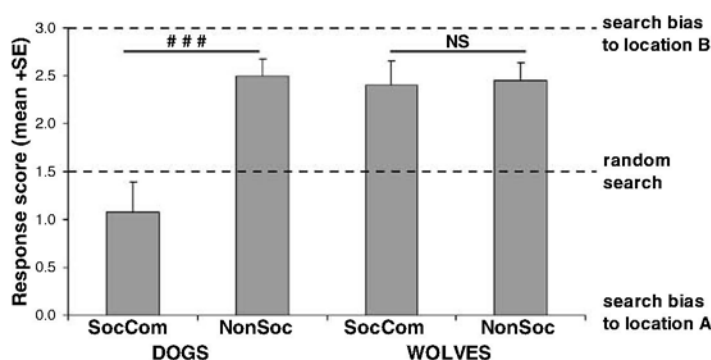
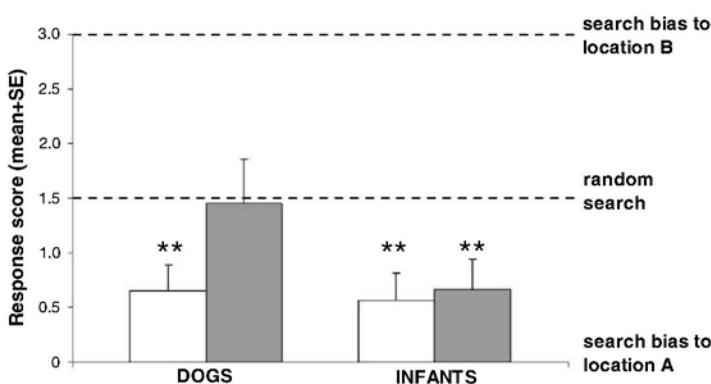


Fig. 3. Dogs and 10-month-old infants respond differentially to the switch of the experimenter in the ostensive communicative hiding context. Left (white) columns indicate correct responses (mean + SE) with the same experimenter [data from experiment 1 (dogs) and from Topál *et al.* 2008 (infants)] in B tests. Right (gray) columns indicate the performance during the B trials in the experimenter-switch condition (experiment 3). ** $P < 0.02$, one-sample t test, in comparison with the success rate expected by random search (0.5 times three B trials).



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Supporting Online Material

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28 May 2009; accepted 21 July 2009

10.1126/science.1176960

Positive Interactions Promote Public Cooperation

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The public goods game is the classic laboratory paradigm for studying collective action problems. Each participant chooses how much to contribute to a common pool that returns benefits to all participants equally. The ideal outcome occurs if everybody contributes the maximum amount, but the self-interested strategy is not to contribute anything. Most previous studies have found punishment to be more effective than reward for maintaining cooperation in public goods games. The typical design of these studies, however, represses future consequences for today's actions. In an experimental setting, we compare public goods games followed by punishment, reward, or both in the setting of truly repeated games, in which player identities persist from round to round. We show that reward is as effective as punishment for maintaining public cooperation and leads to higher total earnings. Moreover, when both options are available, reward leads to increased contributions and payoff, whereas punishment has no effect on contributions and leads to lower payoff. We conclude that reward outperforms punishment in repeated public goods games and that human cooperation in such repeated settings is best supported by positive interactions with others.

The prisoners' dilemma illustrates the tension between private and common interest. Two people can choose between cooperation and defection. If both cooperate, they get more than if both defect, but if one person defects while the other cooperates, the defector gets the highest payoff and the cooperator gets the lowest. In a one-time prisoners' dilemma game, it is therefore in each person's interest to defect. However, if pairs of people play the game repeatedly, it is no longer obvious that defection promotes the defector's private interest, because today's defection may lead the opponent to defect in the future. Under suitable conditions, such direct reciprocity can support cooperation (1–6). Even if people play different opponents in every round, my opponent tomorrow may condition her choice on my play today. Such indirect reciprocity can also sustain cooperation (7, 8). Direct and indirect reciprocity represent fundamental aspects of human interaction, both in evolutionary history and in

modern life: Repetition is often possible, and reputation is usually at stake.

The public goods game is a prisoners' dilemma with more than two people (9). Typically, there is a choice of how much to contribute to a common pool, which then benefits all participants equally. The maximum payoff for the group is achieved if everyone contributes the full amount, but free riders increase their own payoff by withholding their contribution and still benefiting from the public pool. All of us are engaged in many public goods games, on both large and small scales. For example, reducing CO₂ emissions by driving fuel-efficient cars and minimizing waste is a global public goods game. On a more local level, public goods games include volunteering on school boards or town councils and helping to maintain the roads and fire department in your city, as well as cleaning your dishes at home and doing your share of work at the office.

It has been suggested that costly punishment can uphold cooperation in public goods games (10–12). People are willing to pay a cost for others to incur a cost. Typically, such punishment is directed toward free riders and therefore could be a deterrent for defection (13–15). One problem with punishment is that it generates a social loss by reducing both players' payoffs. This effect, however, could be small if sanctions are used rarely, such that in the long run punishment increases net payoffs by discouraging free riding (16), or if punishments are merely symbolic (17–21). Another

problem is that punishment is sometimes used by free riders against cooperators, either randomly or as an act of revenge (22–25). Moreover, the extent to which punishment is perceived as justified can greatly affect the response of those who have been punished (26). These observations question the proposal that costly punishment is an ideal force for promoting cooperation (12). More generally, the substantial literature emphasizing the beneficial effects of material and symbolic rewards and the negative effects of sanctions on interpersonal relationships (27–31) casts doubt on whether the threat of costly punishment provides the most appropriate incentive for cooperation.

In this study, we demonstrate that it is not costly punishment that is essential for maintaining cooperation in the repeated public goods game but instead the possibility of targeted interactions more generally. In the normal repeated public goods game, if one person lowers his contribution, then I cannot directly reciprocate against this person. I could also lower my contribution, but this action harms everyone in the group. Ultimately, this leads to a decline in cooperation. Therefore, we consider public goods games in which after each round there is also the possibility of targeted interactions with other individuals in the group. One such interaction is costly punishment, but another is costly rewarding, as captured by the standard prisoners' dilemma game. In this scenario, I can reward people who have contributed in the public goods game with cooperation but punish free riders with defection.

In the course of daily life, people are always involved in both public and private interactions. Opportunities exist for mutually beneficial trade, as well as harmful punishment. My behavior toward others is affected by their previous decisions, both in the private and public domain. If I resent my neighbor's gas-guzzling SUV, I could exercise costly punishment by slashing his tires. Conversely, I could be extra helpful to my other neighbor who just bought a low-emission vehicle. Punishment is destructive and carries the risk of retaliation by those who have been punished. This is particularly true in situations where, unlike in most laboratory studies, interactions are not anonymous. Without the cover of anonymity, it seems probable that people would be less inclined to punish and more likely to reward. Here, we ask whether rewards can lead to cooperation in the repeated public goods game.

A total of 192 subjects participated in our study at the Harvard Business School Computer Lab for Experimental Research (32). Subjects

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interacted anonymously via computer screens in groups of four. Subjects were told that they would interact with the same three people for the whole session. We performed one control experiment and three treatments.

In the control experiment, subjects play several rounds of a standard public goods game in groups of four (16 control groups). In each round, subjects receive 20 monetary units and decide how much to contribute to the public pool and how much to keep for themselves. The contributions are multiplied by 1.6 and split evenly among the four group members. Subjects are not told the total number of rounds. For a discussion of end-game effects, see (32).

In the three treatments, each public goods game is followed by a second stage, which allows for responses targeted at each other group member. These targeted interactions have different forms in the three treatments (32). In the first treatment (PN, 10 groups), subjects can punish or do nothing. In the second treatment (RN, 11 groups) subjects can reward or do nothing. In the third treatment (RNP, 11 groups) subjects can choose among reward, nonaction, and punishment.

Figure 1A shows the average contribution to the public goods game in each round. Consistent with previous findings, we observe that the average contribution declines in the control experiment but stays high in the punishment treatment, PN. However, we also observe that the two other treatments, RN and RNP, are equally effective in maintaining cooperation in the public goods game. Therefore, it is not punishment per se that is important for sustaining contributions but rather the possibility of targeted interactions. This option is present in all three treatments but absent in the control experiment.

Figure 1B shows the percentage of the maximum possible payoff achieved in each round. The maximum payoff is obtained for full cooperation in the public goods game, no punishment use in the PN treatment, and full rewarding in the targeted rounds of the RN and RNP treatments. All three treatments in which targeted interactions are possible outperform the control after an initial period of adjustment. We again find that reward works as well as punishment, with no significant difference in percentage of maximum possible payoff between the three targeted treatments.

Figure 1C shows the average payoff in each round, summed over the public goods game and the targeted interaction. In the RN and RNP treatments, there is the possibility of generating additional income during the targeted interactions. Thus, it follows naturally from Fig. 1B that the reward treatments, RN and RNP, generate larger absolute payoffs than the punishment-only treatment, PN. Groups that have the opportunity to reward do better than groups that can only punish. The point we want to make is this: If several targeted interactions can promote cooperation in the public goods game, then those that generate additional positive payoff will result in the best outcomes.

Figure 2 shows the frequency of reward and punishment in each targeted round. We see that both options are used. We also see clear changes in punishment and reward use over time. In the PN and RNP treatments, punishment use decreases over time. In the RN and RNP treatments, reward use increases over time. The latter finding suggests that rewarding is stable and does not decay over time, in contrast to findings in a setting where the possibility for direct reciprocity was limited

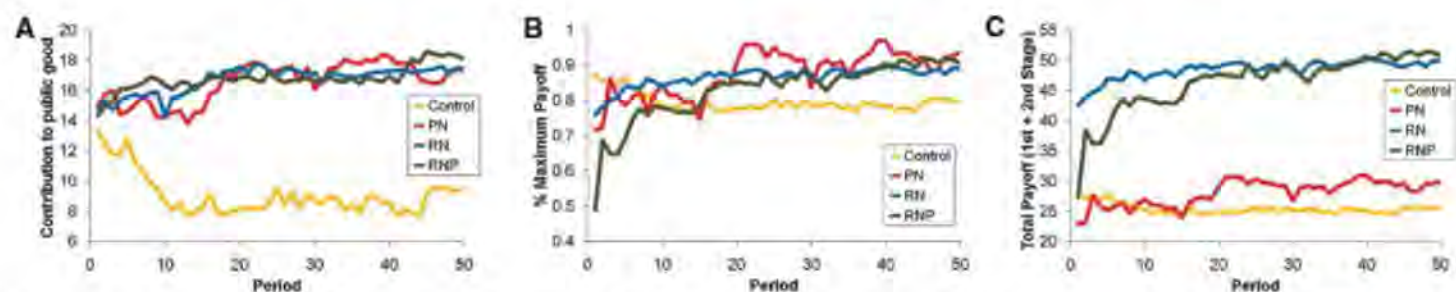


Fig. 1. Mean contribution to the public good (A), percentage of maximum possible payoff (B), and mean payoff (C) over 50 rounds of play in the control (yellow), PN (red), RN (blue) and RNP (green) experiments. All three treatments with targeted reciprocity succeed equally well at increasing contributions and percentage of maximum possible payoff relative to the control, and thus the reward treatments RN and RNP result in significantly higher actual payoffs than the punishment treatment PN. All data are analyzed at the level of the group to account for interdependence of outcomes for members of a given group. (A) Sign-rank test comparing contributions in round 1 versus round 50: Control, $P =$

0.028, decrease; PN, $P = 0.18$, no change; RN, $P = 0.036$, increase; RNP, $P = 0.033$, increase. (B) Rank sum comparing percentage of maximum possible payoff in the second half of the game: PN versus control, $P = 0.013$, PN higher; RN versus control, $P = 0.048$, RN higher; RNP versus control, $P = 0.023$, RNP higher; PN versus RN, $P = 0.67$; PN versus RNP, $P = 0.46$; RN versus RNP, $P = 0.40$. (C) Rank sum comparing mean payoff in the second half of the game: PN versus control, $P = 0.013$, PN higher; RN versus control, $P < 0.001$, RN higher; RNP versus control, $P = 0.001$, RNP higher; PN versus RN, $P = 0.001$, RN higher; PN versus RNP, $P = 0.005$, RNP higher; RN versus RNP, $P = 0.40$.

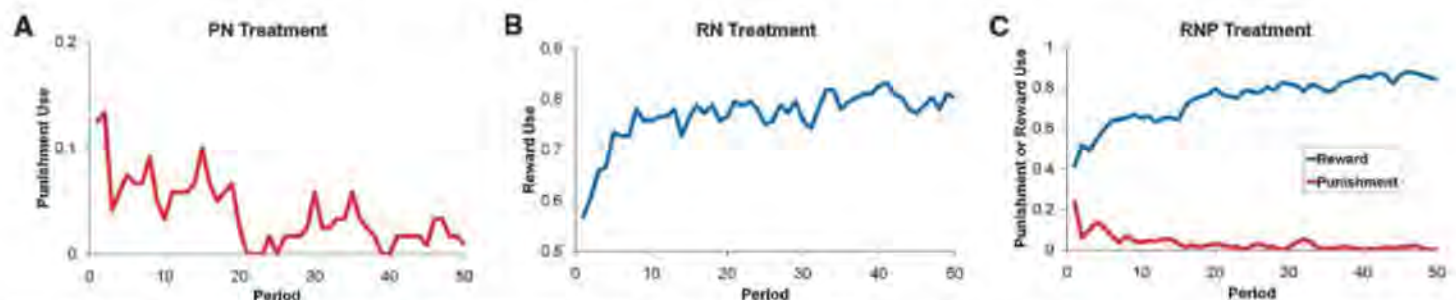


Fig. 2. Frequency of punishment use (red) decreases and reward use (blue) increases over 50 rounds of play in the PN (A), RN (B), and RNP (C) treatments. All data are analyzed at the level of the group to account for interdependence of outcomes for members of a given group. (A) Sign-rank comparing punishment use in rounds 1 and 50: $P = 0.12$; comparing rounds 1 to 5 with rounds 46 to 50: $P = 0.073$, decreases; comparing rounds 1 to 10 with rounds 41 to 50: $P = 0.037$, decreases. (B) Sign-rank comparing reward use in rounds

1 and 50: $P = 0.018$, increases; comparing rounds 1 to 5 with rounds 46 to 50: $P = 0.033$, increases; comparing rounds 1 to 10 with rounds 41 to 50: $P = 0.075$, increases. (C) Sign-rank comparing move use in rounds 1 and 50: R, $P = 0.007$, increases; P, $P = 0.007$, decreases; comparing rounds 1 to 5 with rounds 46 to 50: R, $P = 0.006$, increases; P, $P = 0.004$, decreases; comparing rounds 1 to 10 with rounds 41 to 50: R, $P = 0.006$, increases; P, $P = 0.009$, decreases.

by shuffling player identifiers from round to round (33).

If positive reciprocity alone (RN) and negative reciprocity alone (PN) both increase contributions relative to the control, one might think that putting the two together (RNP) would be best, as found previously in a two-player proposer-responder game (34). However, the RNP setting shows that positive and negative reciprocity cannot be combined in an additive way. The average contribution and percent of maximum possible payoff in RNP are not significantly different from that of RN or PN (Fig. 1). Moreover, the average total payoff in RNP is not significantly different from RN, but is significantly higher than PN.

We can also see that when both options are available, groups that reward more earn higher payoffs while groups that punish more earn lower payoffs (Fig. 3, A and B). It could be that the groups who punished more heavily did so merely because they had the bad luck of containing more free riders, not because they had a greater tendency to punish. However, we see a similar pattern when we examine the probability to punish or reward based on the contribution level in the public goods game (first-order conditional reward and punishment strategies). Groups that are more likely to reward average or above-average contributors achieve significantly higher average contributions (Fig. 3C). Conversely, the tendency to punish low contributors has no effect on contributions (Fig. 3D). As a result, choosing to reward good behavior leads to significantly higher payoffs (Fig. 3E), whereas opting to punish free riders results in marginally lower payoffs (Fig. 3F), because punishing is costly but ineffective in the RNP treatment. When both options are possible, positive reciprocity trumps negative reciprocity for improving contributions in the public goods game and total payoffs.

We have shown that several types of targeted interactions can stabilize contributions in the repeated public goods game. Most previous experiments have focused on punishment and examined situations where subjects cannot track the identity of other group members who punished them. In such settings, typically the groups are changed or the identities of group members are reshuffled in every round. Subjects are often informed about the total amount of punishment they received, but not from whom the punishment came. These designs reduce or eliminate effects of reputation, as well as retaliation by those who have been punished.

Previous studies of reward versus punishment in such settings that limit direct reciprocity have found rewards to be largely ineffective (33–36). In our experiment, however, which is based on repeated interactions where future consequences discipline your actions today, reward is as effective as punishment. We think that this type of truly repeated interaction plays an important role in the study of human behavior. Our ancestors lived in small groups where repeated interactions were common, reputation was often at stake, and the identities of those that chose to punish or

reward were usually known (37). Such concerns are still relevant in today's world, because many of our actions have future consequences. This is particularly true in the context of our most important interactions with family members, friends and co-workers. Thus, although we sometimes find ourselves in anonymous one-time interactions where costly punishment might be more effective than reward, the importance of rewards in repeated interactions should not be overlooked. Moreover, other tools for encouraging cooperation exist beyond monetary punishments and rewards, such as ostracism (19) and appeals to normative values (27). The relative effectiveness of such additional mechanisms merits further study.

Indirect reciprocity settings can also stabilize cooperation in the public goods game (38, 39). Such experiments differ from ours in several ways

and were not designed to directly compare punishment and reward. Moreover, in these studies, subjects are informed about their partner's full history of past play with all previous partners. In our study, we showed that private pairwise interactions, where players do not know what happens in games between others, can still stabilize contributions. It is useful to know that full transparency, which is hard to achieve in the real world, is not necessary for targeted interactions to promote public cooperation.

A common argument for the evolution of costly punishment rests on group selection (40). If group selection is evoked as a mechanism for human cooperation, however, then it is important to note that groups that find positive interactions to maintain cooperation in the public goods game will outperform groups that use costly punish-

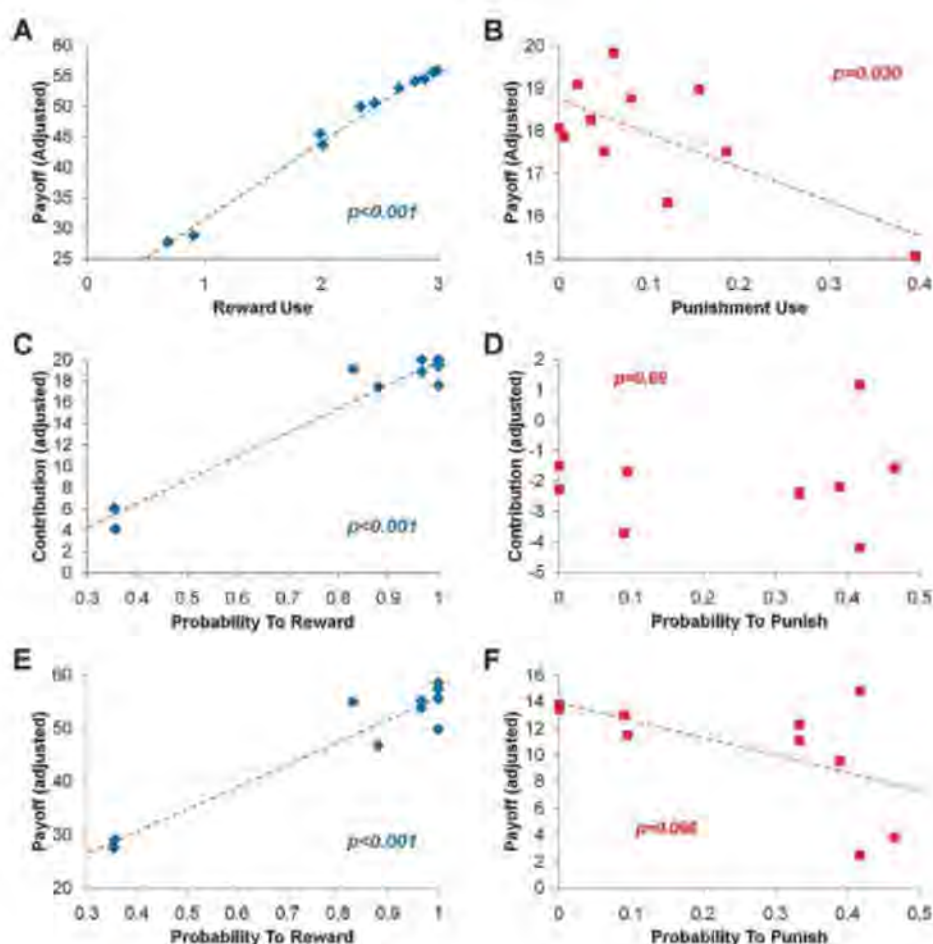


Fig. 3. Mean payoff over the 50 rounds of play in the RNP treatment increases with reward frequency (A) (Tobit, slope = 12.7, $P < 0.001$) and decreases with punishment frequency (B) (Tobit, slope = -7.9 , $P = 0.030$). Mean contribution to the public good increases with the average probability to reward players who contribute equal to or greater than the group average contribution (C) (Tobit, slope = 22.2, $P < 0.001$) and is not significantly related to the probability to punish below-average contributors (D) (Tobit, slope = 1.1, $P = 0.69$). Mean payoff increases with the probability to cooperate with players who contribute equal to or greater than the group average contribution (E) (Tobit, slope = 41.8, $P < 0.001$) and decreases with the probability to punish below average contributors (F) (Tobit, slope = -13.2 , $P = 0.066$). Data are analyzed at the level of the group to account for the interdependence of outcomes for members of a given group. To correctly visualize the results of a multiple regression analysis, the y axis of each panel is adjusted to account for the variation explained by the independent variable shown in the opposing panel [punishment in (A), (C), and (E) and reward in (B), (D), and (F)]. See (32) for regression tables, axis adjustment details, and further statistical analysis.

ment. Moreover, cross-cultural differences have been observed in antisocial punishment, where low contributors punish high contributors (24). Although antisocial punishment is rare among subjects from the United States or the United Kingdom, it is quite common in countries such as Greece and Oman. Thus, although punishment may eventually improve payoffs in long games using subjects from the United States or the United Kingdom, as in the present study and (16), this is almost certainly not the case in areas where antisocial punishment is common. Instead, antisocial punishment could easily result in significantly lower payoffs.

Although we have documented the effects that bilateral punishment and reward can have on multilateral cooperation, our experiment does not allow us to look at the reverse effect. That is, we do not know whether there is more or less bilateral reward or punishment than there would have been if the subjects had not also been engaged in the public goods game. This aspect of linking together different games has received little attention in the experimental literature and deserves further study.

Sometimes it is argued that it is easier to punish people than to reward them. We think this is not the case. Life is full of opportunities for mutually beneficial trade, as well as situations where we can help others, be they friends, neighbors, office mates, or strangers. We regularly spend time and effort, as well as money, to assist people around us. This assistance can be minor, like helping a friend to move furniture, working extra shifts to cover for an ill co-worker, or giving directions to a tourist. It can also be more important, like recommending a colleague for promotion or speaking out to support a victim of discrimination. These sorts of productive interactions are the building blocks of our society and should not be disregarded.

Our study allows a direct comparison of various kinds of targeted interactions on promoting public cooperation in repeated games. We find that reward is as effective as punishment in maintaining contributions to the public good. However, although punishment is costly for both parties, reward creates benefit and thus results in higher total payoffs. Furthermore, when both punish-

ment and reward are possible, positive reciprocity supersedes negative reciprocity, and punishing results in lower group-level benefits. Although punishment may outperform rewards in one-time anonymous interactions, our findings suggest that positive reciprocity should play a more important role than negative reciprocity in maintaining public cooperation in repeated situations. Imagine that there are groups where people either use punishment or reward to induce public cooperation. Which groups will receive the highest payoffs, and therefore which incentive system is optimal? The results are unequivocal: Rewards produce better outcomes than punishment in repeated settings. These findings highlight the importance of developing opportunities for constructive interactions between individuals, to help us prevent tragedies of the commons.

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Supporting Online Material

www.sciencemag.org/cgi/content/full/325/5945/1272/DC1
Materials and Methods
SOM Text
Figs. S1 to S3
Tables S1 to S3
References

8 June 2009; accepted 23 July 2009
10.1126/science.1177418



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2010 "Session I" Meeting Schedule and Preliminary Programs

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Gordon Research Conferences: "Session I" 2010 Preliminary Programs (continued)

The list of meetings, topics and speakers begins below (discussion leaders are noted in italics). Gordon Research Seminars (GRS) are listed below their associated Gordon Conference.

ANGIOTENSIN

Molecular Basis For Action, Dysfunction And New Therapeutic Approaches

Feb 21-26, 2010

Ventura Beach Marriott

Ventura, CA

Chair: Walter G. Thomas

Vice Chair: Kathryn Sandberg

- **Angiotensin Antagonism - From Snake Venom to Vaccine**
(*Ernesto L. Schiffrin* / Nancy Brown / Juerg Nussberger)
- **A Decade of ACE2 - Cloning to Clinic**
(*Tony Turner* / David Brenner / Gavin Oudit)
- **Genetic Modifiers and the Expression of Complex Phenotypes**
(*Jose Krieger* / Jon Seidman / John Mullins / Nancy Cox)
- **Receptor Signaling and Disease**
(*Laszlo Hunyady* / Brad Berk / Marta Ruiz-Ortega / Gervaise Loirand)
- **Diabetic Complications and the RAS**
(*Mark Cooper* / Rudy Bilous / Janos Peti-Peterdi / Atsuhiko Ichihara)
- **Stretch and Antibody Mediated Activation of AT1 Receptors**
(*Sadashiva Kamik* / Issei Komuro / Thomas Gudermann / Yang Xia)
- **Opportunities Based on Selectivity of the N and C Active Sites in ACE**
(*Ken Bernstein* / Oscar Carretero / Ed Sturrock / Ken Bernstein)
- **Angiotensin and Cell Programming**
(*Dominik Müller* / Elias Zambidis / Aurelie Contrepas)
- **Local RAS and New Applications**
(*Richard Re* / Ryuichi Morishita / Andrew Allen)
- **Young Investigator Presentations**
(*Kathryn Sandberg*)
- **Reflections and Predictions**
(*Ken Baker* / Kevin Catt / Ron Smith)
- **Late Breaking Additions**
(*Tom Coffman*)

NEW! ANTIBODY BIOLOGY & ENGINEERING From Basic Mechanisms To Antibody-Based Therapeutics

Mar 7-12, 2010

Ventura Beach Marriott

Ventura, CA

Chairs: E. Sally Ward & Davinder Gill

Vice Chair: Derry Roopenian

- **Keynote Presentation: B Cell Biology**
(*Donald Capra* / Klaus Rajewsky / Jason Cyster)

- **Antibody Repertoires and Diversification Mechanisms**
(*Fred Alt* / Michael Neuberger / Nina Papavasiliou / Patrick Wilson / James Marks)
- **Molecular Recognition by Antibodies and Fcγ Receptors**
(*Dennis Burton* / Ian Wilson / Dinakar Salunke / Mark Hogarth)
- **Functional Aspects of Fcγ Receptors and Complement**
(*Jenny Woolf* / Raphael Clynes / Louis Weiner / John Cambier)
- **FcRn and IgG Dynamics**
(*John Desjarlais* / Richard Blumberg / Inger Sandlie / William Dall'Acqua)
- **Imaging of Antibodies and Antibody Related Processes**
(*Raimund Ober* / Diane Lidke / Michael Carroll / Anna Wu)
- **B Cell Tolerance**
(*Betty Diamond* / Huub Schellekens / Eric Meffre / David Nemazee)
- **Antibody Fragments and Novel Protein Scaffolds**
(*Paul Carter* / Andreas Plückthun / Dane Wittrup / Kerry Chester / Richard Begent / Patrick Bäuerle)
- **Therapeutic Antibodies**
(*Andrew Chan* / Philip Thorpe / Wayne Marasco / Steve Jacobsen)

AUTOPHAGY IN STRESS, DEVELOPMENT & DISEASE

Apr 25-30, 2010

Il Ciocco Hotel and Resort

Lucca (Barga), Italy

Chair: Noboru Mizushima

Vice Chairs: Vojo Deretic & Sharon A. Tooze

- **Autophagy from Model Organisms to Humans**
(*Zvulun Elazar* / Thomas Neufeld / Yoshinori Ohsumi / Ana Maria Cuervo)
- **Regulation of Autophagy**
(*Patrice Codogno* / Beth Levine / Guido Kroemer / Adi Kimchi / Francesco Cecconi)
- **Autophagy Signaling**
(*David Rubinsztein* / Thomas Neufeld / Do-Hyung Kim / Junying Yuan)
- **Autophagy Machinery**
(*Suresh Subramani* / Zvulun Elazar / Jae Jung / Tamotsu Yoshimori / Douglas Green)
- **Origin of the Autophagosome**
(*Eeva-Liisa Eskelinen* / Nicholas Ktistakis / Fulvio Reggiori)
- **Selective Autophagy**
(*William Dunn* / Masaaki Komatsu / Terje Johansen / Richard Youle / Matthias Peter)

- **Metabolic Diseases & Cancer**
(*Guido Kroemer* / Aimee Edinger / Eileen White / Marco Sandri / Hirotaka Watada)
- **Innate & Adaptive Immunity**
(*Beth Levine* / Ludger Klein / Skip Virgin / Shoichiro Kurata)
- **Aging & Neurology**
(*Junying Yuan* / David Rubinsztein / Anne Simonsen / Ralph Nixon)



BIOGENIC HYDROCARBONS & THE ATMOSPHERE

May 23-28, 2010

Les Diablerets Conference Center

Les Diablerets, Switzerland

Chairs: Jose D. Fuentes & Paolo Ciccioli

Vice Chairs: Christine Wiedinmyer & Paul Palmer

- **BVOCs and the Atmosphere: Past and Present**
(*Guenther Seufert* / Reinhold Rasmussen / Jos Lelieveld)
- **Biosynthesis and Emission of BVOCs from Plants**
(*Mary Anne Carroll* / Tom Sharkey / Russell K. Monson / Jurgen Kesselmeier)
- **Ecological Functions of BVOCs Emitted by Plants**
(*Dorothea Toll* / Francesco Loreto / Natalia Dudareva)
- **Atmospheric Reactivity of BVOCs**
(*William Brune* / John Orlando / Robert Griffin / Laurens Ganzeveld)

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- **Early Career Scientists I**
(Rainer Steinbrecher)
- **BVOC's and Climate Change**
(Paulo Artaxo / Marku Kulmala / Meinrat Andreae / Joice Penner)
- **Early Career Scientists II**
(Allison L. Steiner)
- **BVOC and Industrial Processes**
(Thomas E. Pierce / Jorg Peter Schnitzler / Franco Biasioli / Elena E. Stashenko / Arlene Fiore)
- **Future Directions**
(Nicholas C. Hewitt / Ted Turlings / Jochen Rudolph)



NEW! BIOLOGY & PATHOBIOLOGY OF THE CORNEA

Advances In Cornea, Conjunctiva, Meibomian And Lacrimal Gland Research

Mar 7-12, 2010

Four Points Sheraton / Holiday Inn Express
Ventura, CA

Chairs: Shukti Chakravarti & Steven E. Wilson
Vice Chairs: James Jester & Suzanne Fleiszig

- **Development, Structure and Function**
(John R. Hassell / David E. Birk / Keith Meek)
- **Genetics, Genomic & Proteomic Approaches**
(Alexander V. Ljubimov / Akhilesh Pandey / Sara S. Murray / Albert Jun)
- **Barrier Functions and Secretion**
(Ilene K. Gipson / Bogi Andersen / Stephen C. Pflugfelder)
- **Wound Healing Response and Cell Signaling**
(James D. Zieske / Robert M. Lavker / Mary A. Stepp)

- **Microbial and Viral Infections**
(Sally S. Atherton / Linda D. Hazlett / Todd P. Margolis)
- **Immune Response, Inflammation and Angiogenesis**
(Reza Dana / Shigeru Kinoshita / Eric Pearlman)
- **Transplantation and Immune Modulation**
(Jerry Y. Niederkorn / Robert L. Hendricks / Junko Hori / Pedram Hamrah)
- **Surgery Bioengineering and Biomimetics**
(Donald T. Tan / Virender Sangwan / Jennifer H. Elisseeff)
- **Stem Cell Biology and Applications**
(Graziella Pellegrini / Yann Barrandon / James L. Funderburgh)

NEW! BIOLOGY OF ACUTE RESPIRATORY INFECTION

Mar 21-26, 2010

Four Points Sheraton / Holiday Inn Express
Ventura, CA

Chair: Jay K. Kolls

Vice Chairs: Alice Prince & Kanta Subbarao

- **Acute Respiratory Infections in 2010**
(Joseph Mizgerd / A.D.M.E. Osterhaus / Craig Rubens)
- **Epidemiology and Biology of Acute Viral Infections**
(A.D.M.E. Osterhaus / Jeffrey Taubenberger / Ann Falsey / Gregory C. Gray / Fernando Pollack)
- **The Microbiome and Acute Respiratory Infections**
(Ann Falsey / Martin J. Blaser / Homer Boushey)
- **Mucosal Immunity in the Lung**
(Jeffrey Weiser / Bart Lambrecht / Bruce Beutler / Joseph Mizgerd / Nicole Baumgarth)
- **Bacterial Infections of the Lower Respiratory Tract**
(Frank R. DeLeo / Michael R. Wessels / Jeffrey Weiser / Victor Nizet)
- **Staphylococcus Aureus Infections**
(Victor Nizet / Henry F. "Chip" Chambers / Frank R. DeLeo / Michael Otto)
- **Fungal Pneumonias**
(James Gern / Gordon Brown / Luigina Romani / Chad Steele)
- **Co-infections and Pathogenesis**
(Chad Steele / James Gern / Tina Hartert / Guy Boivin)
- **Prevention/Treatment of Acute Respiratory Infections**
(Maria Zambon / Pradeep Singh)

BIOMOLECULAR INTERACTIONS & METHODS

Protein Interaction Dynamics: Theory, Method & Practice

Jan 17-22, 2010

Hotel Galvez

Galveston, TX

Chairs: Karen Fleming & Gideon Schreiber

Vice Chairs: Linda K. Nicholson & Justin E. Molloy

- **Molecular Network Dynamics**
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- **Macromolecular Machines and Assemblages**
(Keiichi Namba / Robert Sauer / Kiyoshi Nagai)
- **Advances in Biophysical Methods**
(Colin Kleanthous / Marius Clore / Helen Saibil)
- **Intrinsically Unstructured Proteins and Conformational Diversity**
(Vince Hilser / Terry Oas / David Eliezer)
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(David Warshaw / Justin Molloy / Joseph Puglisi)
- **Advances in Computational Methods**
(Gideon Schreiber / Angel Garcia / Brian Kuhlman)
- **Kinetics & Thermodynamics**
(Madeline Shea / Linda Nicholson / Enrique de la Cruz)
- **Keynote Presentation: Dynamics of Ribosome Assembly, in vitro and in Cells, Using Mass Spectrometry**
(Karen Fleming / Jamie Williamson)

NEW! BIOMOLECULAR INTERACTIONS & METHODS (GRS)

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Hotel Galvez

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Chairs: Ann Marie Stanley & Rachel E. Farrow

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- **Speakers:** To be selected from submitted abstracts
- **Discussion Leaders:** Madeline Shea and Linda K. Nicholson
- **Keynote Speaker:** Carlos Bustamante
- **Mentorship Component:** "Beyond Graduate School", a career panel featuring Linda Nicholson, Enrique De La Cruz, Lumelle Schneeweis, and John Beals

CAROTENOIDS

Jan 17-22, 2010

Ventura Beach Marriott
Ventura, CA

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Vice Chairs: Eleanore T. Wurtzel &
Xiang-Dong Wang

- **Photosynthesis**
(Kris Niyogi)
- **Biosynthesis and Regulation in Plants**
(Giovanni Giuliano /
Manuel Rodriguez-Concepcion /
Joseph Hirschberg)
- **Carotenoid Transport and Metabolism /
Apocarotenoids**
(William Blamer / Loredana Quadro /
Earl Harrison / Georg Lietz)
- **Genomics, Modeling, and Systems Biology**
(Donald Bryant / Peter Bramley)
- **Carotenoids in Eye Health**
- **Biological Actions of Carotenoids /
Apocarotenoids**
(Johannes Von Lintig / Lewis Rubin /
Fuzhi Lian / Harro Bouwmeester /
Kazuo Miyashita)
- **Carotenoids and Oxidation: Chronic
Disease Prevention**
(John Erdman / Luigi Ferrucci /
Steven Clinton / Paola Palozza)
- **Metabolic Engineering of Provitamin A
Carotenoids**
(Richard Sayre / Steven Schwartz /
Paul Christou)
- **Keynote Presentation**
(George Britton / Paul Bernstein)

CHEMOTACTIC CYTOKINES

May 30 - Jun 4, 2010

Il Ciocco Hotel and Resort
Lucca (Barga), Italy

Chair: Gerry Graham

Vice Chair: Antal Rot

- **Keynote Presentation**
(Gerry Graham / Alberto Mantovani)
- **Chemokines and Disease I**
(Phil Murphy / Eleanor Fish / Sam Hwang /
Sunhil Ahuja / Mauro Teixeira / Don Cook)
- **Chemokines and Stem Cells**
(Albert Zlotnik / Sara Rankin / Erez Raz /
Alain Ghysen)
- **Chemokines and Disease II**
(Barrett Rollins / Fran Balkwill /
Richard Ransohoff / Sergio Lira /
Antonella Viola / Bernhard Homey)
- **Atypical Chemokine Receptors**
(Amanda Proudfoot / Antal Rot / Rob Nibbs /
Massimo Locati / Marcus Thelen)
- **Chemokines and Leukocyte Movement**
(Ronen Alon / Andy Luster / Gwen Randolph /
Charles MacKay / David Jackson)

- **Chemokines and Secondary Lymphoid
Function**
(Graham Anderson / Reinhold Foerster /
Bill Agace / Michael D Gunn / Reina Mebius)
- **Chemokine and Chemokine Receptor
Structure Studies**
(Tracey Handel / Chris Overall /
Brian Volkman / Marc Parmentier)
- **Chemokine Therapeutics**
(Tom Schall / Matt Walters / Andreas Kungl /
Nic Fischer)

COLLOIDAL, MACROMOLECULAR & POLYELECTROLYTE SOLUTIONS Dynamics In Complex Fluids And Solutions

Feb 21-26, 2010

Four Points Sheraton / Holiday Inn Express
Ventura, CA

Chair: Lynn M. Walker

Vice Chairs: Norman Wagner & Andrey Dobrynin

- **Colloidal Interactions**
(Steve Granick / Monica Olvera de la Cruz)
- **Particle Assembly**
(Darrell Velegol / Ilona Kretzschmar /
Keith Johnston / Marjolein Dijkstra)
- **Manipulation of Colloids with Flow**
(Patrick Doyle / Wilson Poon)
- **Attractive Colloids and Gels**
(Dimitris Vlassopoulos / Victor Breedveld /
Surita Bhatia)
- **Ions in Polymers**
(Thomas Epps / Nitash Balsara /
Robert Weiss)
- **Complex Fluid Structure Control**
(Walter Richtering / Patrick Spicer /
Carlos Rinaldi / Julian Eastoe)
- **Macromolecules in Complex Environments**
(Michael Rubinstein / Christos Likos /
Shiyong Liu)
- **Macromolecules in Electric Fields**
(Gary Slater / James Schneider /
Christian Holm)
- **Future Trends**
(Eric Kaler / Benjamin Chu)

COMPOSITES

Toward The Development Of Sustainable & Nanotechnology Inspired Multi-Functional Composites

Jan 17-22, 2010

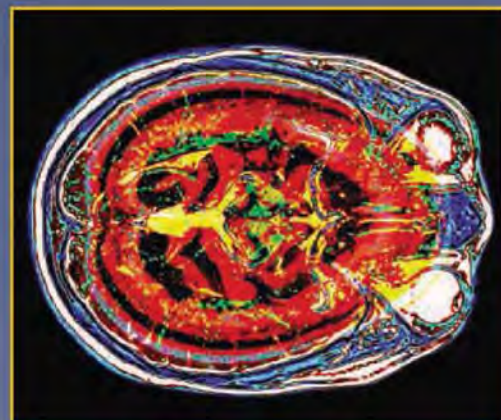
Four Points Sheraton / Holiday Inn Express
Ventura, CA

Chair: Gale Holmes

Vice Chair: Richard Wool

- **Keynote Presentation: Nanocomposites -
An Industrial Perspective**
(Tie Lan)

- **Mechanical Properties of Nanocomposites
(the Nano-Effect)**
(Nikhil Koratkar / Eyal Zussman /
Anthony Kinloch)
- **A Processing Look at Nanocomposite
Formation**
(Donald R. Paul / Evangelos Manias)
- **CNT Nanocomposites (Conductivity,
Dispersion, and Other Issues)**
(Stephen J. Eichhorn / Jaime W. Grunlan /
Ton Peijs / Ian Kinloch)
- **The Future and Nano-Environmental Health
and Safety**
(Frank Jones)
- **Novel Nanostructured Materials:
Microstructure & Mechanical Behavior**
- **Theory of Nanocomposite Formation &
Confinement Effects**
(James L. Thomson / Jack Douglas /
Sindee Simon)
- **Multi-Functional Nanocomposites**
(Asa H. Barber / Yiu-Wing Mai /
Pulickel M. Ajayan / Lawrence T. Drzal)
- **Recyclable Composites (for a Sustainable
Future)**
(David Plackett / Jeffrey Gilman)



CRANIOFACIAL MORPHOGENESIS & TISSUE REGENERATION

Apr 11-16, 2010

Il Ciocco Hotel and Resort
Lucca (Barga), Italy

Chair: Paul Trainor

Vice Chair: Robert Maxson

- **Neural Crest: Formation, Migration,
Differentiation**
(Paul Trainor / Marianne Bronner-Fraser /
Roberto Mayor)
- **Placodes: Formation and Differentiation**
(Marianne Bronner-Fraser / Andrea Streit /
Irma Thesleff)
- **Evolution and Development**
(Chuck Kimmel / Gerhard Schlosser /
Michael Depew)
- **Tissue Interactions and Morphogenesis**
(Andrea Streit / Eldad Tzahor / Fred Relaix /
Yang Chai)
- **Signaling and Communication**
(Michael Depew / Gary Landreth / Filippo Rijli)

- **Craniofacial Disorders and Syndromes I**
(Andrew Wilkie / Max Muenke / Ralph Marcucio / Stan Lyonnet)
- **Craniofacial Disorders and Syndromes II**
(Rob Maxson / Andy Copp / Danny Huylebroeck)
- **Stem Cells, Regeneration and Tissue Engineering**
(Irma Thesleff / Paul Sharpe / Pam Yellick)
- **Keynote Presentations: Evolution, Development and Disease**
(Paul Trainor / Charles Kimmel / Andrew Wilkie)

NEW! CRANIOFACIAL MORPHOGENESIS & TISSUE REGENERATION (GRS)

Apr 10-11, 2010

Il Ciocco Hotel and Resort

Lucca (Barga), Italy

Chairs: Samantha A. Brugmann & Rebecca J. Richardson

The Gordon-Kenan Research Seminar on Craniofacial Morphogenesis & Tissue Regeneration is a unique forum for graduate students, post-docs, and other scientists with comparable levels of experience and education to present and exchange new data and cutting edge ideas. The focus of this meeting is to explore the mechanisms underlying the evolution, development, patterning, disease, and regeneration of the tissues that make up the craniofacial complex. Topics of interest include, but are not limited to: neural crest and placode ontogeny, signaling pathway and gene regulation, tissue-tissue interactions, development of skeletal tissues, organogenesis; including tooth and palate morphogenesis, stem cell biology and tissue morphogenesis and regeneration.

- **Speakers:** To be selected from submitted abstracts
- **Discussion Leaders:** Paul Trainor, Sally Moody, and Nathan M. Young



DNA DAMAGE, MUTATION & CANCER

Mar 21-26, 2010

Ventura Beach Marriott

Ventura, CA

Chair: Joann B. Sweasy

Vice Chair: Karen M. Vasquez

- **Keynote Presentations: Genomic Predictors of Responses to Chemotherapy / MicroRNAs and Cancer**
(Leona Samson / Frank Slack)
- **Endogenous DNA Damage**
(Sheila David / Takehiko Nohmi / John Tainer / Susan Wallace)
- **Properties of DNA and Mutagenesis**
(Jaqueline Barton / Jean-Sebastien Hoffman / Kristin Eckert)
- **Conformational Dynamics and Polymerase Fidelity**
(Catherine Joyce / Suse Broyde / F. Peter Guengerich / Samuel Wilson)
- **Chromatin Structure and DNA Repair**
(Jac Nickoloff / Andrew Xiao)
- **Translesion Synthesis: Stochastic or Ordered?**
(Wei Yang / Graham Walker / Alan Lehman / Myron Goodman)
- **Mutagenesis and Cancer**
(Bradley Preston / Polina Schberkova / Carlo Maley / Lawrence Loeb)
- **Transcriptional and Translational Mutagenesis**
(Andres Aguilera / Paul Doetsch / Bradley Cairns / Hani Zaher)
- **DNA Repair and Cancer Therapy**
(Peter Glazer / Helen Pownall-Worms / Jeremy Rich / Joanne Weidhaas)

ELECTROCHEMISTRY

Jan 10-15, 2010

Four Points Sheraton / Holiday Inn Express

Ventura, CA

Chair: Stephen E. Creager

Vice Chair: Daniel A. Scherson

- **Keynote Presentation: Electrochemistry and Alternative Energy**
(Stephen Creager / Nathan Lewis)
- **Catalysis / Nanoelectrochemistry**
(Carlos Cabrera / Andrzej Wieckowski / Jens Nørskov / Stan Whittingham)
- **Bioelectrochemical Energy Transduction**
(Ken Nealon / Leonard Tender)
- **Electrochemical Energy Storage I**
(Bryan Pivovar / Linda Nazar / Grant Smith / Joykumar Thokchom)
- **Electrochemical Energy Storage II**
(John Kerr / Esther Takeuchi / Angela Belcher)
- **Young Investigator Presentations**
(Takashi Ito / Jill Venton / Ryan O'Hayre / Janine Mauzeroll)
- **Bioelectrochemistry**
(Tony Guiseppi-Elie / Jim Rusling / Zuzanna Siwy)

- **Electrochemical Information Storage and Motion Actuation**
(Rick McCreery / Werner Kuhr / Michael Kozicki / Joe Wang)

NEW! ELECTROCHEMISTRY (GRS)

Jan 9-10, 2010

Four Points Sheraton / Holiday Inn Express
Ventura, CA

Chair: Ryan J. White

The Gordon-Kenan Research Seminar on Electrochemistry is a unique forum for graduate students, post-docs, and other scientists with comparable levels of experience and education to present and exchange new data and cutting edge ideas. The focus of this meeting is on a broad range of electrochemical phenomena and their applications in energy and information storage, motion actuation, and (bio)chemical sensing and analysis.

- **Speakers:** To be selected from submitted abstracts
- **Discussion Leaders:** Debra Rolison, Kevin W. Plaxco and Zuzanna S. Siwy
- **Keynote Speaker:** Richard Van Duyne

ENVIRONMENTAL ENDOCRINE DISRUPTORS

May 30 - Jun 4, 2010

Les Diablerets Conference Center

Les Diablerets, Switzerland

Chairs: Andreas Kortenkamp & R. Tom Zoeller

Vice Chair: Andrea C. Gore

- **From Short-Term High-Dose Studies to Long-Term Low-Dose Effects?**
(Andreas Kortenkamp / Craig Steinmaus / Laura Vandenberg)
- **Endocrine Disruptors and the Male Reproductive System I**
(Niels E Skakkebaek / Henrik Leffers / Martin Cohn / Ioanna Katsiadaki)
- **Endocrine Disruptors and the Male Reproductive System II**
(Shanna Swan / Elizabeth Hill)
- **Endocrine Disruptors and Female Reproductive Health**
(Louis Guillette / Teresa K Woodruff / Paul Fowler)
- **Obesity and the Environment**
(Jerry Heindel / Tania Adam / Bruce Blumberg)
- **Hormonal Neoplasia**
(Andreas Kortenkamp / Gail Prins)
- **Hormonal Neoplasia and Cancer Theories - Problems with the Paradigm?**
(Carlos Sonnenschein / Paolo Vineis)
- **Thyroid Disruption and Environmental Pollutants**
(R. Tom Zoeller / Veerle Darras / Joanne Rovet / Eric Mondie)
- **Science and its Implications for Chemicals Regulation**
(Andrea Gore / Earl Gray)

NEW! FIBROBLAST GROWTH FACTORS IN DEVELOPMENT & DISEASE

Mar 14-19, 2010

Four Points Sheraton / Holiday Inn Express
Ventura, CA

Chairs: Sabine Werner & Moosa Mohammadi
Vice Chair: Jeremy E. Turnbull

- **Keynote Presentations: The FGF Family**
(Sabine Werner, Moosa Mohammadi / Judith Abraham / Andrew Beenken / Steven Klierer)
- **Biochemistry of FGFs and FGF Signalling**
(Jeremy Turnbull / Claudio Basilico / David Fernig / John Heath / Akira Imamoto / Walter Nickel / Jeremy Turnbull)
- **Interactions of FGFs with Other Signalling Pathways**
(Gail Martin / Blanche Capel / David Ornitz / Irma Thesleff)
- **FGFs in Development**
(David Ornitz / Nobuyuki Itoh / Gail Martin / Olivier Pourquie / Deborah Yelon / Yoseph Yost)
- **FGFs in the Nervous System**
(Klaus Unsicker / Juha Partanen / Klaus Unsicker / Steve Wilson)
- **Endocrine FGFs**
(Steven Klierer / Makoto Kuro-O / Beate Lanske / Wallace McKeehan / Yo-ichi Nabeshima)
- **FGFs in Developmental/Genetic Disease**
(Bradley Olwin / Marja Hurley / Nelly Pitteloud / Andrew Wilkie)
- **FGFs in Tissue Repair, Regeneration and Remodelling**
(Andrew Wilkie / Saverio Bellusci / George Cotsarelis / Bradley Olwin / Michael Simons)
- **FGFs in Cancer**
(Judith Abraham / Jing Qing / Axel Ullrich / Fen Wang)

GENES & BEHAVIOR

Integration

Mar 14-19, 2010

Ventura Beach Marriott
Ventura, CA

Chair: David F. Clayton

Vice Chair: Catharine H. Rankin

- **Adaptive Nature of Learning**
(Louis Lefebvre, Sara Shettleworth / Tadeusz Kawecki / Alcino Silva)
- **Young Investigator Presentations**
(Robert Anholt, Constance Scharff)
- **Integrating "Brain" into Genes and Behavior**
(Alison Bell, Jonathan Flint / Paul Sternberg / Catherine Dulac)
- **The Lasting Imprint of the Battle Between the Sexes**
(Jane Hurst, Robert Williams / David Crews / Art Arnold / Molly Cummings / Elena Jazin)

- **Gene/Behavior Studies of Natural Populations**
(Amy Toth, Darlene Francis / Clyde Hertzman / Hopi Hoekstra)
- **Changing Perspectives: from Units to Networks to Landscapes**
(Ralph Greenspan / Trudy Mackay / Erich Jarvis / Charlie Whitfield / Allen Moore)
- **Epigenetic Transmission of Behavioral Traits**
(Russ Fernald, Per Jensen / David Goldman / Michael Meaney)
- **Behavioral Development & Disorders: Evolutionary & Mechanistic Perspectives**
(Abraham Palmer, Justin Rhodes / Ulrike Heberlein / Hannah Monyer / David Skuse)
- **Boundaries and Forces in Behavioral Evolution**
(Marla Sokolowski, Johan Bolhuis / Bernard Crespi / Fred Nijhout)

NEW! GENES & BEHAVIOR (GRS)

Mar 13-14, 2010

Ventura Beach Marriott
Ventura, CA

Chairs: Seth A. Ament & Margaret S. Ferris

The Gordon-Kenan Research Seminar on Genes & Behavior is a unique forum for graduate students, post-docs, and other scientists with comparable levels of experience and education to present and exchange new data and cutting edge ideas. The focus of this meeting is on recent advances in understanding the relationships between genes and behavior, bringing together researchers using both traditional "behavioral models" and "genetic models," as well as researchers studying human behavior genetics.

- **Speakers:** To be selected from submitted abstracts
- **Discussion Leaders:** John Hildebrand, Catherine Rankin and Hopi Hoekstra
- **Keynote Speakers:** David Clayton and Jonathan Flint

GLYCOLIPID & SPHINGOLIPID BIOLOGY

Feb 7-12, 2010

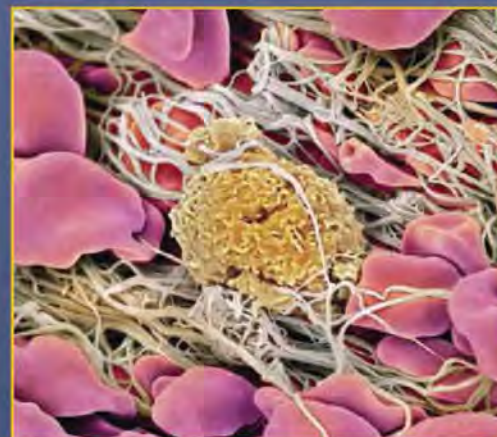
Ventura Beach Marriott
Ventura, CA

Chair: Lina M. Obeid

Vice Chair: Yasuyuki Igarashi

- **Biophysics and Biomembranes**
(Howard Riezman, Toshihide Kobayashi / Alicia Alonso / Alessandro Prinetti / Liana Casquinha da Silva)
- **Glycolipid and Sphingolipid Biochemistry: Enzymes of Sphingolipid Metabolism**
(Mariana Nikolova-Karakashian, Konrad Sandhoff / Teresa Dunn / Joost Holthuis / Makoto Ito / Hiroko Hama)

- **Glycolipid and Sphingolipid-Based Chemistry and Structural Biology Approaches**
(Gerhild van Echten-Deckert, Tony Futerman / Gemma Fabrias / James A. Shayman / Hiroko Ikushiro)
- **Cell Biology: Transport and Cell Regulation**
(Walt Holleran / Antonella De Matteis / Neale Ridgway / Marion B. Sewer / Akio Kihara)
- **Translational Aspects of Sphingolipid Biology I: Cancer Biology and Therapy**
(Richard Kolesnick, Amy S. Paller / Besim Ogretman / Myles Cabot / Tzipora Goldkorn / Charles Swanton)
- **Translational Aspects of Sphingolipid Biology II: Metabolic Diseases**
(Mark Kester, Koichi Furukawa / Ronald L. Schnaar / Wayne I. Lencer / Hugh J. Willison / Glyn Dawson)
- **Translational Aspects of Sphingolipid Biology III: Lipid Storage Diseases**
(Frances Platt, Edward Schuchman / Emyr Lloyd-Evans / Rahm Gummuluru / Roger Sandhoff)
- **Lipid Mediated Signaling**
(Sarah Spiegel, Thierry Levade / Timothy Hla / Stewart Pitson / Sue Pyne / Antonio Gomez-Munoz)
- **Poster Presentations**
(Maurizio Del Poeta, Ashley Cowart)
- **Sphingolipidomics and Model Systems**
(Al Merrill / Robert Dickson / Julie Saba / Richard Proia / Mitsutoshi Setou / M. Cameron Sullards)



IMMUNOCHEMISTRY & IMMUNOBIOLOGY

May 16-21, 2010

Les Diablerets Conference Center
Les Diablerets, Switzerland

Chair: Caetano Reis e Sousa

Vice Chair: Cynthia J. Guidos

- **Innate Immunity and Inflammation**
(Ruslan Medzhitov / Max Cooper / Yasmine Belkaid / Jurg Tschopp)
- **Lymphoid Stress-Surveillance Responses**
(Adrian Hayday / Mitch Kronenberg / Angela Santoni / Eric Vivier)

- **Leukocyte Development**
(Cynthia Guidos / Jim P. Di Santo / Harinder Singh / Meinrad Busslinger)
- **Antigen Presentation and Lymphocyte Activation**
(Doreen Cantrell / Sebastian Amigorena / Mark Davis / Michael Reth)
- **Immune Cell Interactions**
(Marc Jenkins / Ron Germain / Ellen Robey / Michael Sixt)
- **Generation of Effector Lymphocytes**
(Gitta Stockinger / Steve Reiner / Carola Vinuesa / Ed Palmer)
- **Immune Regulation and Dysfunction**
(Diane Mathis / Dan Littman / Federica Sallusto / Sasha Rudensky)
- **Immunity to Infections**
(Antonio Lanzavecchia / Andreas Radbruch / Annette Oxenius / Andrea Cooper)
- **The Immune System at Mucosal Surfaces**
(Andrew MacPherson / Fiona Powrie / Lora Hooper / Bart Lambrecht)



ISOTOPES IN BIOLOGICAL & CHEMICAL SCIENCES
Isotopic Probes For Mechanisms In The Chemical And Life Sciences
 Feb 14-19, 2010
 Hotel Galvez
 Galveston, TX
 Chair: John P. Richard
 Vice Chair: Vernon E. Anderson

- **Isotopes in Medicine**
(Ronald Kluger / Henri Brunengraber / J. Thomas Brenna)
- **Heavy Atom Isotope Effects**
(Nigel Richards / Justine Roth / Alvan Hengge / Scott Strobel)
- **Computational Modeling of Isotope Effects**
(Piotr Paneth / Vicente Moliner / Jiali Gao)
- **Isotope: From Model Studies to Enzyme Catalysts**
(Barbara Schowen / Hans Limbach / Andrew Bennet / Ian Williams / Ildiko Kovach / Daniel Quinn)
- **Isotopic Probes in Genomic Enzymology**
(Debra Dunaway-Mariano / Tadhg Begley / John Gerlt)

- **Isotopic Probes of Organic Reaction Mechanisms**
(Dean Tantillo / Matthew Meyer / Weston Borden / Charles Perrin)
- **Proton Transfer Reactions**
(Daniel O'Leary / Sharon Hammes-Schiffer / Kevin Peters)
- **Isotopic Probes of Enzymatic Reaction Mechanisms**
(Andrew Murkin / Paul Cook / Paul Fitzpatrick / Amnon Kohen)
- **Isotopes at the Frontiers of Science**
(V. Jack Shiner / Daniel Singleton / Vern Schramm)

LIGAND RECOGNITION & MOLECULAR GATING

May 16-21, 2010
 Il Ciocco Hotel and Resort
 Lucca (Barga), Italy
 Chair: Carola Hunte
 Vice Chair: Kenton J. Swartz

- **Structures and Mechanism of Secondary Transporters**
(Etana Padan / Eric Gouaux [Keynote] / Olga Boudker / Peter Henderson / Christopher Miller)
- **Ligand-Gated Ion Channels**
(Carola Hunte / Raimund Dutzler / Toshi Kawate)
- **Voltage-Gated Ion Channels and Intercellular Communication**
(Kenton Swartz / Baron Chanda / David Clapham / Tomitake Tsukihara)
- **Passages Through the Outer Membrane**
(Susan Buchanan / Jeff Abramson / Gerhard Wagner)
- **Membrane-Protein Lipid Interactions**
(Anthony Lee / Bert van den Berg)
- **Nucleotide-Coupled Transporters**
(Joao Morais-Cabral / Hassane Mchaourab)
- **New Methods Probing Membrane Protein Structures and Conformations**
(Bert Poolmann / Marc Baldus / Hartmut Oschkinat / Daniel Nietlispach)
- **G Protein Coupled Receptors and Interaction with G Proteins and Ligands**
(Gebhard Schertler / Klaus-Peter Hofmann / Brian Kobilka / Jorg Standfuss)

MARINE NATURAL PRODUCTS

Feb 28 - Mar 5, 2010
 Ventura Beach Marriott
 Ventura, CA
 Chair: Guy T. Carter
 Vice Chair: Amy E. Wright

- **Inspirational Structures of Bioactive Marine Natural Products**
(John Cardellina / Phil Crews / Jun'ichi Kobayashi)

- **Harnessing the Biosynthetic Capacity of Microbial Symbionts**
(Bill Gerwick, Russell Hill / Joern Piel / Margo Haygood / Eric Schmidt)
- **Chemical Biology & Mechanism of Action**
(Mark Hamann, Chris Ireland / Charles Boone / Carole Bewley / Dale Nagle)
- **Genomic Approaches to Marine Natural Product Discovery**
(Russel Kerr, Brad Moore / Greg Challis / Paul Jensen / Rolf Muller / Michael Fischbach)
- **New Microbial Sources for Marine NP Drug Discovery**
(Bill Baker, Bill Fenical / Hans-Peter Fiedler / Jeffrey Janso / John Waterbury)
- **Drugs from the Sea: Beyond Scientific Curiosity**
(Raymond Andersen, Ted Molinski / Paul Wender / Ian Paterson / Ron Quinn)
- **Converting the Uncultured: Novel Sources of MNP's**
(Jon Clardy, Paul Jensen / Stephen Giovannoni / Luis Maldonado)
- **Young Investigator Presentations**
(Phil Crews, Julia Kubanek / Pieter Dorrestein / Alessandra Eustaquio / Hendrik Leusch / Kerry McPhail / David Rowley / Ryan Van Wagoner)

NEW! MECHANICAL SYSTEMS IN THE QUANTUM REGIME

Mar 21-26, 2010
 Hotel Galvez
 Galveston, TX
 Chairs: Keith C. Schwab & David McClelland
 Vice Chairs: Tobias J. Kippenberg & Konrad W. Lehnert

- **Macro-Opto-Mechanics**
(Yanbei Chen / Jack Harris / Nergis Mavalvala)
- **Micro-Opto-Mechanics**
(Khaled Karrai / Oskar Painter / Hong Tang / Ho Bun Chan)
- **Coupling Atoms to Mechanics**
(Mukund Vengalattore / Philipp Treutline / Pierre Meystre)
- **New Theory**
(Aash Clerk / Florian Marquardt / Ignacio Wilson-Rae / Gerard Milburn)
- **Entanglement with Mechanics**
(Eyal Buks / Pierre-Francois Cohadon)
- **Gravitation and Mechanics**
(Carlton Caves / Aharon Kapitulnik / David Blair)
- **Molecular-Scale Mechanics**
(Jim Hone / Herre Van der Zandt / Adrian Bachtold)
- **Advances in Electronic Detection**
(Jared Hertzberg / Alex Rimberg / John Teufel / Eva Weig)
- **Dissipation and Decoherence**
(Sheila Rowan / Miles Blencowe / Jeevak Parpia)

Gordon Research Conferences: "Session I" 2010 Preliminary Programs (continued)

METALS IN BIOLOGY

Jan 31 - Feb 5, 2010

Four Points Sheraton / Holiday Inn Express
Ventura, CA

Chair: John D. Lipscomb

Vice Chair: William B. Tolman

- **Understanding the Chemistry of Biology**
(Edward Solomon / Kenneth Karlin / Frank Neese)
- **Diagnosis, Drugs, and Delivery**
(Stephen Lippard / Thomas Meade / Pradip Mascharak / Christoph Fahrni / Janet Morrow)
- **Activating O₂ Without Getting Burned: Nature's Toolkit**
(Lawrence Que, Jr. / Lindsay Eltis / Brian Fox / Sonya Franklin)
- **Designing Metalloproteins and Bio-Directed Materials**
(Yi Lu / Yoshihito Watanabe / Chuan He / Trevor Douglas / Shana O. Kelley)
- **Clusters in Catalysis**
(Stephen Cramer / Oliver Einsle / John Peters / José Moura)
- **Metal Selection and Transport**
(Catherine Drennan / Nigel Robinson / Collin Stultz / Peng Chen / Amy Rosenzweig)
- **Spectroscopy, Theory, and Applications**
(Serena DeBeer George / Britt Hedman / Christopher Cramer / Nicolai Lehnert)
- **Many Faces of Metalloporphyrins**
(John Dawson / Thomas Poulos / Shunichi Fukuzumi / Syun-Ru Yeh / Jennifer DuBois)
- **Joint Session with Gordon Research Seminar**
(William Tolman / Karl Wieghardt)

BIOINORGANIC CHEMISTRY (GRS)

Feb 4-7, 2010

Four Points Sheraton / Holiday Inn Express
Ventura, CA

Chair: Kathryn E. Cole

Vice Chair: Christine M. Phillips

The Gordon Research Seminar on Bioinorganic Chemistry is held in conjunction with the Metals in Biology GRC and is a unique forum for graduate students, post-docs, and other scientists with comparable levels of experience and education to present and exchange new data and cutting edge ideas. Speakers will be selected from submitted abstracts.

- **Joint Session with Metals in Biology GRC followed by GRS Poster Session**
- **Metal Homeostasis and Transport**
(Pamela Riggs-Gelasco)
- **Biom mineralization and Bioinspired Materials**
(Trevor Douglas)
- **Metalloenzyme Kinetics and Mechanisms**
(Squire Booker)
- **Metals in Medicine and Metallo drug Design**
(Debbie Crans)
- **Biomimetic Renewable Energy**
(Gary Brudvig)

MYELIN

Feb 14-19, 2010

Ventura Beach Marriott
Ventura, CA

Chair: Wendy B. Macklin

Vice Chairs: William D. Richardson & James L. Salzer

- **What are the Obstacles for Validation of Remyelination Therapies?**
(Steven Goldman / Timothy Vartanian)
- **Gene Expression in the Oligodendrocyte Lineage**
(Ben Barres / Ben Emery / David Rowitch / Nicole Schaeren-Wiemers / Mikael Simons / Hauke Werner)
- **Cell Migration in Brain Development**
(Anthony Campagnoni / Shumin Duan / Emma Frost / Oscar Gonzalez Perez / Elek Molnar)
- **Signaling Mechanisms Controlling Myelination**
(Vittorio Gallo / Charles ffrench-Constant / Babette Fuss / Terry Wood)
- **Epigenetic Control of Myelination**
(Patrizia Casaccia-Bonnel / Sjeff Copray / Richard Lu / Mark Mehler / Lucia Notterpek)
- **Axonal Pathology in De/Dysmyelinated Tissue**
(Peter Brophy / Selva Baltan-Tekkok / Siddharthan Chandran / Klaus Nave / Martin Stangel / Bruce Trapp)
- **Remyelination in the Adult Nervous System**
(Catherine Lubetzki / Regina Armstrong / Gareth John)
- **Myelin Involvement in Psychiatric and Neurologic Disorders**
(Gabriel Corfas)
- **Stem Cells vs. Progenitor Cells / Transplantation vs. Endogenous Repair: Which is Most Useful?**
(Ian Duncan / Annick Baron van Evercooren / Jeff Kocsis / Robert Miller / Anne Mother)

NEW ANTIBACTERIAL DISCOVERY & DEVELOPMENT

Mar 14-19, 2010

Hotel Galvez

Galveston, TX

Chairs: George Drusano & Eric D. Brown

Vice Chairs: Christian Hubschwerlen & Hans-Georg Sahl

- **Keynote Presentations: New Hope for New Antibacterial Drugs**
(Trevor Trust / Roberto Kolter)
- **Multidrug Resistance**
(Keith Poole / Shariar Mobashery / Olga Lomavskaya)
- **Pharmacodynamics**
- **Success Stories in Discovery and Development**
(Jared Silverman)
- **The Role of Government**

New Biology

(Claire Poyart / Marvin Whitely / Christopher Whitfield)

New Chemistry

(Justin Nodwell / Brian Bachman)

New Approaches to Antibacterial Discovery

(Bob Hancock / Terry Roemer)

New Approaches to Antibacterial Development



ORIGIN OF LIFE

Jan 10-15, 2010

Hotel Galvez

Galveston, TX

Chair: George E. Fox

Vice Chair: Henderson J. Cleaves

- **Keynote Presentation**
(Gunter Wachterhausen)
- **Deep Carbon Cycle**
(John Baross / George Cody / Tom McCollum / Martin Schoonen / Norman Sleep)
- **Prebiotic Chemistry**
(Steve Benner / Nicolas Giuseppone / Richard Sole / David Deamer / Henderson Cleaves)
- **Extant and/or Ancient Life on Mars?**
(David Desmarais / Bethany Ehlmann / Nicholas Tosca / Jennifer Eigenbrode / Tori Hoehler)
- **Self Replicating Systems**
(Henderson Cleaves / Nicholas Hud / Richard Sole / Eos Szathmari / Andy Ellington)
- **Assembling Primitive Life: New Approaches and Challenges**
(David Deamer / Jan Spitzer / Tetsuya Yomo / Irene Chen / Anthony Forster)
- **Origin and Evolution of Ribosomes**
(George Fox / Ada Yonath / Loren Williams / Hyman Hartmann / Zaida Luthe-Schuleton)
- **Early History of Living Systems**
(Andy Ellington / Arcady Mushegian / Steve Benner / John Baross)

ORIGIN OF LIFE (GRS)

Jan 9-10, 2010

Hotel Galvez

Galveston, TX

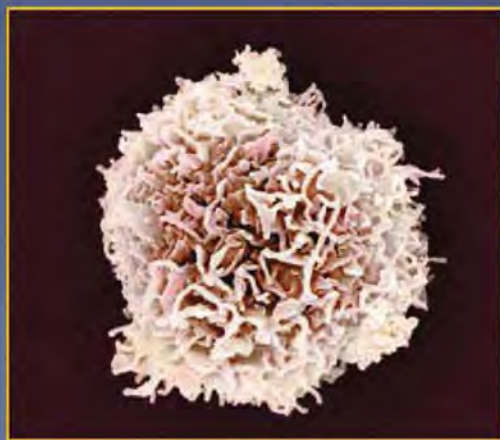
Chairs: Michael P. Callahan & Nicole R. Posth

The Gordon Research Seminar on Origin of Life is a unique forum for graduate students, post-docs, and other scientists with comparable levels

visit the frontiers of science. . . go to a gordon conference! (www.grc.org)

of experience and education to present and exchange new data and cutting edge ideas. The origin of life community encompasses disciplines such as physics, astronomy, chemistry, biology, molecular biology, ecology, geology, and planetary science. Within the origin of life context, new research from the fields of astrochemistry and planetary science, the RNA world, geobiology, genomics and early life will be presented and discussed.

- **Speakers:** To be selected from submitted abstracts
- **Discussion Leaders:** To be selected from submitted abstracts



OXYGEN RADICALS

Mechanisms That Underpin Redox Biology

Feb 7-12, 2010

Four Points Sheraton / Holiday Inn Express
Ventura, CA

Chairs: Garry Buettner & Anthony J. Kettle
Vice Chairs: Neil Hogg & Valerie B. O'Donnell

- **Oxidative Stress, Redox Biology and Disease**
(Balz Frei, Garry R. Buettner / Lawrence J. Marnett / Dean P. Jones)
- **Inflammation and Oxidative Stress**
(Christine C. Winterbourn, Victor Darley-Usmar / William M. Nauseef / Karl-Heinz Krause / Flavia Meotti / Stephan E. Baldus / Eric E. Kelley)
- **Oxidative Events in Cardiovascular Disease**
(Anthony J. Kettle / Jay W. Heinecke / Roland Stocker)
- **Biochemistry of Thiol Oxidation and Thiol Enzymes**
(Neil Hogg, Valerie O'Donnell / Leslie B. Poole / Mark B. Hampton / Regina Brigelius-Flohe / Kenneth D. Tew)
- **Nitrogen Oxides and Hydrogen Sulfide**
(Jon Lundberg / Karl-Heinz Krause / Jack R. Lancaster / Philip K. Moore)
- **Protein Oxidation, Redox Changes in Aging**
(Balaraman Kalyanaraman / Rodney L. Levine / Vadim N. Gladyshev / Rajindar S. Sohal / Kelvin J.A. Davies)

- **Mitochondria, Central to Redox Balance**
(Henry Forman, Dean Jones / Paul S. Brookes / Alicia J. Kowaltowski / Tak Yee Aw)
- **Biomarkers and Methods for Detecting Oxidative Stress**
(Jay W. Heinecke, Roland Stocker / Piepie Ping / Frederick A. Villamena / Jeannette Vasquez-Vivar / C. Alan Butterfield / Victor Darley-Usmar)
- **Keynote Presentation: Superoxide Dismutases, Past, Present and Future**
(Garry R. Buettner / Joe M. McCord)

PEPTIDES, CHEMISTRY & BIOLOGY OF

Feb 28 - Mar 5, 2010

Four Points Sheraton / Holiday Inn Express
Ventura, CA

Chairs: Samuel H. Gellman & Mark R. Spaller
Vice Chairs: Fred R. Naider & Maria A. Bednarek

- **Keynote Presentations: Pushing the Boundaries of Peptide Science**
(Lila Gierasch / Bill DeGrado)
- **Peptide Assembly**
(Amy Keating / Joel Schneider / Dek Woolfson / Sam Stupp)
- **Antimicrobial Peptides**
(Tom Ganz / Yechiel Shai / Jared Silverman)
- **Peptide-Based Medicinal Chemistry**
(Dean Madden / Kurt Deshayes / Les Miranda / Soumitra Ghosh)
- **Peptides & Synthesis**
(Jeff Bode / Ron Raines / Helma Wennemers)
- **Peptide Engineering**
(Beate Kokschi / Ernest Giralt / Paramjit Arora / John Robinson)
- **Biological Applications**
(David Lawrence / Dev Sidhu / Hiroaki Suga)
- **Peptides in Membranes & Physical Analysis**
(Don Engelman / Karen Fleming / Mei Hong / Steve Martin)

PHOTOIONS, PHOTOIONIZATION & PHOTODETACHMENT

Jan 31 - Feb 5, 2010

Hotel Galvez
Galveston, TX

Chair: Albert Stolow
Vice Chair: Danielle Dowek

- **Photodetachment**
(Carl Lineberger / Mark Johnson / Lai-Sheng Wang / Matthias Weber / Andrei Sanov)
- **Photoionization**
(Nora Berrah / Laurent Nahon / Kiyoshi Ueda / Pascal Lablanquie / Erwin Poliakov / Pierre Billaud)
- **Complex Systems I**
(Tom Gallagher / Klaus Müller-Dethlefs / Ed Grant / Paola Bolognesi)

- **Strong Field Ionization**
(Misha Yu. Ivanov / Henrik Stapelfeldt / Thomas Baumert / Andreas Becker / Artem Rudenko / Claus-Peter Schulz / Jochen Mikosch)
- **Theory**
(Tamar Seideman / Todd J. Martinez / Anna Krylov / Faris Gel'mukhanov)
- **Complex Systems II**
(John H.D. Eland / Manfred Faubel / Majed Chergui / Roger Falcone / Oliver Gessner / Michael Schuurman)
- **Keynote Presentation: Chemical Physics**
(Robert E. Continetti / Vince McKoy / Ingo Fischer / Lionel Poisson / Andrey Boguslavskiy)
- **Attosecond Physics**
(Margaret Murnane / Marc Vrakking / Olga Smirnova / Chris Greene / Hans-Jakob Wörner)
- **Photoelectron Angular Distributions**
(Jonathan G. Underwood / Katharine Reid / Robert Lucchese / Mauro Stener / Christer Bisgaard)

PHOTOSENSORY RECEPTORS & SIGNAL TRANSDUCTION

Apr 18-23, 2010

Il Ciocco Hotel and Resort
Lucca (Barga), Italy

Chair: Kevin H. Gardner
Vice Chair: Christian Fankhauser

- **LOV Domains: Structure, Mechanism and Engineering**
(Aba Losi / John Christie / Klaus Hahn / Andreas Möglich)
- **Microbial Rhodopsins**
(Hartmut Luecke / Klaus Gerwert / Peter Hegemann / John Spudich)
- **Phytochrome Structure / Function Relationships**
(J. Clark Lagarias / Lars-Oliver Essen / Keith Moffat / Eberhard Schaefer / Rick Vierstra)
- **Biophysical Methods As Applied To Photosensors**
(Klaas Hellingwerf / Marloes Groot / Wayne Hubbell / Tom Sakmar / Masahide Terazima)
- **Photosensory Components of Biological Clocks**
(Jay Dunlap / Samer Hattar / Amita Seghal / David Somers)
- **BLUF Domains**
(Ilme Schlichting / Carl Bauer / Mineo Iseki)
- **Cryptochromes**
(Alfred Batschauer / Margaret Ahmad / Steve Reppert / Dongping Zhong)
- **Visual Rhodopsins: Models for GPCR Signaling**
(Oliver Ernst / Kevin Ridge / Gebhard Schertler)

- **Phytochrome Signaling**
(Eberhard Schaefer / Joanne Chory /
J. Clark Lagarias / Akira Nagatani)

NEW! PHOTSENSORY RECEPTORS & SIGNAL TRANSDUCTION (GRS)

Apr 17-18, 2010

Il Ciocco Hotel and Resort
Lucca (Barga), Italy

Chairs: Abigail I. Nash & Andreas Möglich

The Gordon-Kenan Research Seminar on Photosensory Receptors & Signal Transduction is a unique forum for graduate students, post-docs, and other scientists with comparable levels of experience and education to present and exchange new data and cutting edge ideas. The focus of this meeting will be on the molecular mechanisms of light perception and signal transduction in photoreceptor proteins. Discussions will include all classes of photoreceptors as well as the recently developed fields of photoreceptor design and biotechnological applications.

- **Speakers:** To be selected from submitted abstracts
- **Discussion Leaders:** Erik Schleicher, Nathan C. Rockwell, Marcela Ávila Pérez and Tilo Mathes

**PINEAL CELL BIOLOGY
Mechanisms Of Circadian Rhythmicity
And Melatonin Action**

Feb 7-12, 2010

Hotel Galvez
Galveston, TX

Chair: David R. Weaver

Vice Chair: Debra J. Skene

- **Regulation of Melatonin Production**
(Helena Illnerova / Anthony K. Ho /
Jorg H. Stehle)
- **Circadian Clocks in the Retina**
(Gianluca Tosini / Joseph C. Besharse /
P. Michael Iuvone / Chad R. Jackson /
Cara M. Altimus)
- **New Developments in Pineal Developmental Biology**
(Maria Seron-Ferre / Joshua T. Gamse /
Margaret J. Ochocinska / Diego Bustos /
Martin F. Rath)
- **Circadian Clocks, Melatonin and Metabolism I**
(Carla B. Green / Eleanor M. Scott /
Hindrik Mulder / Akhilesh B. Reddy /
David A. Bechtold)
- **Cellular Mechanisms of Melatonin Action**
(Elizabeth S. Maywood /
Paula A. Witt-Enderby / Vincent M. Cassone /
Gabriella Gobbi)

- **Circadian Clocks, Melatonin and Metabolism II**
(David G. Hazlerigg / Takashi Yoshimura /
Sandrine M. Dupre / Timothy J. Bartness /
Valerie Simonneaux)
- **Melatonin, Circadian Clocks, and CNS Diseases**
(Sarah H. Elsea / James Olcese)
- **Melatonin Effects on Sleep and Circadian Rhythms**
(Debra J. Skene / Irina V. Zhdanova /
Phyllis C. Zee / Charmane I. Eastman /
Kenneth P. Wright, Jr.)
- **When Light Interrupts the Night: Dying for Darkness**
(David C. Klein / Jennifer A. Evans /
Alec J. Davidson / Richard G. Stevens /
Till Roenneberg)

PLANT-HERBIVORE INTERACTION

Feb 21-26, 2010

Hotel Galvez
Galveston, TX

Chair: Heidi M. Appel

Vice Chair: Colin M. Orians

- **New Perspectives in Plant Herbivore Interaction**
(Heidi Appel / May Berenbaum)
- **Herbivores in Complex Communities**
(Susan Hartley / Ilka Feller / Mat Vanderliff /
Todd Palmer / Jennifer Rudgers)
- **Evolution of Herbivore Responses to Plant Chemistry**
(Heidi Appel / Denise Dearing /
Caroline McBride)
- **Plant Defensive Chemistry - Action & Detoxification**
(Jonathan Gershenzon /
Juha-Pekka Salminen / William Foley /
John Tewksbury / Martin Heil)
- **Evolution of Plant and Insect Defense: Glucosinolates**
(Arthur Zangerl / Christopher Wheat /
Daniel Kliebenstein)
- **Interaction Models: From Ecology to Genes**
(Anurag Agrawal / Jordi Bascompte /
Nico Bluthgen / David Weston /
Sharon Strauss)
- **Extended Phenotypes and Evolution**
(Marcel Dicke / Thomas Whitham /
John Thompson)
- **Integrating Below Ground Interactions**
(Nicole van Dam / Mark Hunter / Mattias Erb /
Melissa Mitchum / Katrin Meyer)
- **New Perspectives on Invasive Species**
(Colin Orians / Mirka Macel / Kenneth Raffa)



PLASMINOGEN ACTIVATION & EXTRACELLULAR PROTEOLYSIS

Feb 14-19, 2010

Four Points Sheraton / Holiday Inn Express
Ventura, CA

Chairs: Thomas H. Bugge & Vincent Ellis

Vice Chair: Bradford S. Schwartz

- **Thrombolysis, Fibrinolysis & Hemostasis**
(Nuala Booth / Bruce Beutler [Keynote] /
Ed Plow / Wolfram Ruf)
- **CNS Function and Dysfunction**
(Katerina Akassoglou / Katerina Akassoglou /
Dan Lawrence / Sid Strickland /
J. Steven Jacoben / Rob Medcalf)
- **Cardiovascular Health and Disease**
(Qingyu Wu / David Dicheck / Qingyu Wu /
Kathy Hajjar)
- **Infection, Inflammation and Immunity**
(David Ginsburg / David Ginsburg /
Jay Degen / Aime Zaas / Frank Castellino /
Mark Walker)
- **Tissue Homeostasis & Regeneration**
(Toni Antalis / Toni Antalis /
Pura Munoz-Canoves / Andreas Janecke)
- **Stem Cell Biology & Haematopoiesis**
(Karin Finberg / Marc Tjwa / Birgitte Rønø /
Peter Andreasen)
- **Late Breaking Topics from Abstracts**
(Vincent Ellis, Bradford Schwartz)
- **Tumor Biology and Angiogenesis**
(Sharon Stack / Sharon Stack / Jim Quigley /
Francesco Blasi / Steve Gonias /
Michael Ploug)
- **Metabolism and Obesity**
(Daniel Lawrence / Dudley Strickland /
Joyce Chan)

**NEW! PLASMINOGEN ACTIVATION & EXTRACELLULAR PROTEOLYSIS (GRS)
Extracellular Proteases And Their Regulation:
New Functions In Development And Disease**

Feb 13-14, 2010

Four Points Sheraton / Holiday Inn Express
Ventura, CA

Chairs: Roman Szabo, Dimitrios Davalos &
Daniel H. Madsen

The Gordon-Kenan Research Seminar on Plasminogen Activation & Extracellular Proteolysis is a unique forum for graduate students, post-docs, and other scientists with comparable levels

of experience and education to present and exchange new data and cutting edge ideas. The focus of this meeting is on the basic and clinical research in the field of pericellular proteolysis in general, and plasminogen activation in particular. The meeting seeks to attract young scientists to present their work on the molecular mechanism of pericellular proteolysis, its function in tissue development, homeostasis, and disease, and on the development of new, protease-based therapies for the treatment of disorders of cardiovascular and central nervous systems, as well as cancer.

- **Speakers:** To be selected from submitted abstracts
- **Discussion Leaders:** Thomas H. Bugge and Sharon Stack
- **Keynote Speaker:** Benjamin Cravatt, "Activity-Based Proteomics - Applications for Enzyme and Inhibitor Discovery"



PROTEIN COFACTORS, RADICALS AND QUINONES

Jan 24-29, 2010

Four Points Sheraton / Holiday Inn Express
Ventura, CA

Chair: Britt-Marie Sjöberg

Vice Chair: Joseph T. Jarrett

- **Cross-Linked Cofactors**
(Judith P. Klinman / Victor L. Davidson / Richard Magliozzo)
- **Electron Transfer in Proteins**
(Daniel G. Nocera / Harry B. Gray / Nigel S. Scrutton)
- **Ribonucleotide Reductases**
(JoAnne Stubbe / Astrid Gräslund / Per E.M. Siegbahn)
- **Protein Radical Mechanisms**
(Brian M. Hoffman / Kurt Warncke)
- **Radical SAM Proteins**
(Diana M. Downs / Joan B. Broderick / Squire J. Booker / Joseph T. Jarrett)
- **Hydrogenases**
(Bärbel Friedrich / Marc Fontecave)
- **B₁₂ Enzymes and Riboswitches**
(Ruma Banerjee / Adam Roth)

PROTEIN FOLDING DYNAMICS

Jan 10-15, 2010

Ventura Beach Marriott

Ventura, CA

Chair: Susan Marqusee

Vice Chair: Dave Thirumalai

- **Keynote Presentations: Theoretical and Experimental Approaches to Protein Folding**
(Harold Scheraga / Charlie Brooks / Bill Eaton)
- **Navigating the Energy Landscape**
(Sheena Radford / Doug Barrick / Dan Raleigh / Peter Wolynes / Mikael Oliveberg)
- **Molecular Machines: Dynamics and Function**
(Jose Onuchic / Klaus Schulten / Changbong Hyeon / Andreas Mastoushek)
- **Fluctuations in the Native State**
(Ken Dill / Phil Geissler / Hue Sun Chan / Jane Dyson / Ivet Bahar)
- **From Evolution to Pathways and Design**
(Jane Clarke / Eugene Shakhnovich / Tobin Sosnick / Frances Arnold)
- **Folding in vivo and Off the Ribosome**
(Joan Shea / Vijay Pande / Judith Frydman / George Lorimer / Johannes Buchner)
- **Pushing the Limits**
(Matthias Reif / Julio Fernandez / Ruxandra Dima)
- **Misfolding and Aggregation**
(Jay Winkler / Carol Hall / Yuji Goto / John Straub / Rob Tycko)
- **Protein Energetics and Cosolvents**
(Walter Englander / Rohit Pappu / Ruhong Zhou / George Makhataдзе)

PROTEIN FOLDING DYNAMICS (GRS)

Jan 9-10, 2010

Ventura Beach Marriott

Ventura, CA

Chairs: Riina Tehver & Lorna Dougan

The Gordon Research Seminar on Protein Folding Dynamics is a unique forum for graduate students, post-docs, and other scientists with comparable levels of experience and education to present and exchange new data and cutting edge ideas. The focus of this meeting is to enhance students' and postdocs' background on current techniques, theories and models of protein folding.

- **Speakers:** To be selected from submitted abstracts
- **Discussion Leaders:** Susan Marqusee, Lorna Dougan and Dave Thirumalai

NEW! PROTEIN TRANSPORT ACROSS CELL MEMBRANES

Mar 7-12, 2010

Hotel Galvez

Galveston, TX

Chairs: Reid Gilmore & Tassos Economou

Vice Chairs: Kenneth Cline & Matthias Mueller

- **Protein Import Into Mitochondria**
(Klaus Pfanner / Carla Koehler / Agnieszka Chacinska / Trevor Lithgow)
- **Translocation Through SEC YEG/Sec61 Complex**
(Linda Randall / Arnold Driessen / Shu-ou Shan / Tom Rapoport / Roland Beckmann)
- **Biogenesis of the Peroxisome**
(Suresh Subramini / Ralf Erdmann / Richard Rachubinski / Yukio Fujiki)
- **ER and Periplasm Associated Degradation Pathways**
(Art Johnson / Michael Erhmann / Hidde Ploegh / Randy Hampton)
- **Structural Biology of Protein Transport**
(Harris Bernstein / Babis Kalodimos / Natalie Strynadka / Robert Keenan)
- **Nonconventional Protein Transport Pathways**
(Ross Dalbey / Ana Maria Cuervo / Boris Striepen / Jeff Cox)
- **Integration of Membrane Proteins**
(Nica Borgese / Manu Hegde / David Andrews / Doron Rapaport)
- **Diversity of Prokaryotic Protein Export Pathways**
(Guy Cornelis / Vassilis Koronakis / Francoise Jacob-Dubuisson / Susan Lea)
- **Protein Import Into Chloroplasts**
(Steve Theg / Danny Schnell / Enrico Schleiff / Paul Jarvis)

PROTEOLYTIC ENZYMES & THEIR INHIBITORS

Proteolysis: The Most Important And Ubiquitous Post Translational Modification That Regulates Biology, Life And Death

May 2-7, 2010

Il Ciocco Hotel and Resort

Lucca (Barga), Italy

Chair: Christopher M. Overall

Vice Chair: Klaudia Brix

- **Degradomics**
(Christopher Overall / Kris Gevaert / Anna Prudova / Kevin Wang / Wade Harper)
- **Proteolytic Pathways in Cancer**
(Sharon Stack, Bonnie Sloane / Johanna Joyce / Sharon Stack / Katarina Wolf / Yves Boucher / Yashunori Okada / Izabela Podgorski / Karin List)
- **Proteases in Other Deadly Diseases**
(Agnes Noel / Agnes Noel / Margarete Heck / Nabil Seidah / Marcus Groettrup)

- **Exopeptidases: The Start and End of All Things**
(Xavier Gomis-Rüth / Guy Salvesen / Xavier Gomis-Rüth / F. Xavier Avilés Pulvert / Paul Tempst / Lloyd Fricker / Hans-Ulrich Demuth / Marcin Drag / Tania Baker)
- **Structural Wonders**
(Walter Stocker / Walter Stocker / Ed Sturrah / Irit Sagi / Vern Schramm [Keynote])
- **Protease Pot-Pourri**
(James Whisstock, Jan Potempa / James Whisstock / Edgar Deu Sandoval / Hui Zou / Hans-Ulrich Demuth / Brooke LaFlamme / Kai Grobes / Ky-Anh Nguyen)
- **Blood"y" Proteases**
(Jim Huntington / Jim Huntington / Hans Brandstetter / Sriram Krishnaswamy / Athan Kuliopulos)
- **Quality Control: RIPPed Off**
(Bruno Martoglio, Mike Wolfe / Mike Wolfe / Bruno Martoglio / Mike Wolfe / Yoshinori Akiyama / Colin Adrain / Thierry Meinne / Doron Greenbaum)
- **Proteases that Refresh: Autophagy Cleans Within**
(Klaudia Brix / Adi Kimchi / Urska Repnik / Esther Wong)

RADIATION ONCOLOGY

Translating Scientific Breakthroughs To The Radiation Oncology Patient

Jan 24-29, 2010

Hotel Galvez
Galveston, TX

Chair: William F. Morgan

Vice Chair: Robert Bristow

- **Keynote Presentation 1: Translating Insights from the Cancer Genome into Clinical Practice**
(Joe Gray)
- **Keynote Presentation 2: Radiation Used to Guide Nanoparticle Drug Delivery to the Tumor**
(Dennis Hallahan)
- **Targeting the DNA Damage Response for Cancer Therapy**
(Simon Powell / Mark O'Connor / Sharon Cantor / Tim Kinsella / Conchita Vens)
- **Modulating Radiation Sensitivity by Perturbing Receptor Signaling Pathways**
(Paul Dent / Garth Powis / Daphne Haas-Kogan / Costas Koumenis)
- **Custom Tailoring Radiation Therapy Based on Individual Susceptibilities**
(Bill McBride / Quynh-Thu Le / Kian Ang / Marcel Verheij / Gillies McKenna)
- **System Responses to Radiation that Impact Therapy**
(Mary Helen Barcellos-Hoff / Lynn Hlatky / Andrei V. Gudkov / Katrina Waters)

- **Application of New Modalities / Technologies in Radiation Oncology**
(Richard Kolesnick / Chuan-Yuan Li / Zvi Fuks / Bob Timmerman / Ritsuko Komaki / Hiroshiko Tsujii)
- **The Pros and Cons of Stem Cells in Radiotherapy**
(Charlie Limoli / Wendy Woodward / Frank Pajonk / Joel Greenberger)
- **Anti-Angiogenesis and Radiation Therapy**
(Amato Giaccia / Denise Chen / Chris Willet / Adam Dicker / Dietmar Siemann)
- **Clinical Application of Nanotechnologies in Radiation Therapy**
(Gayle Woloschak / Mohamed Kahn / Nicola Sibson / David Boothman)

SENSORY TRANSDUCTION IN MICROORGANISMS

Jan 24-29, 2010

Ventura Beach Marriott
Ventura, CA

Chair: Ann H. West

Vice Chair: Caroline S. Harwood

- **Diversity in Microbial Chemotaxis**
(Igor Zhulin / Judith P. Armitage / Christopher Rao / Victor Sourjik / Rebecca Parales / Peter N. Devreotes)
- **Receptor Signaling and Architectural Design**
(Barry Taylor / John S. Parkinson / Grant Jensen / John Spudich / Joseph J. Falke / Gerald Hazelbauer)
- **Flagellar Motors**
(Shin-Ichi Aizawa / Tohru Minamino / Kent L. Hill / Michio Homma / Joshua Shaevitz)
- **Intracellular Signaling in Bacteria**
(Frederick Dahlquist / Ann Stock / Ruth Silversmith / Michael Laub / Mark Goulian / Linda McCarter / Urs Jenal)
- **Motility Apparati**
(Nyles Charon / Rasika Harshey / David Blair / Brian R. Crane / Jun Liu)
- **Intracellular Signaling in Eukaryotic Microorganisms**
(Jan Fassler / Richard Calderone / Haruo Saito / Kaz Shiozaki / Katherine Borkovich / William F. Loomis / Alan R. Kimmel)
- **Motility and Spatial Organization**
(Janine Maddock / Lotte Sogaard-Andersen / Zemer Gitai / Chris Janetopoulos / Tām Mignot / David Zusan)
- **Intercellular Communication**
(John R. Kirby / Daniel B. Kearns / Karen L. Visick / Pete Greenberg / Thierry Emonet / Michael Elowitz / Fitnat Yildiz)
- **Pathogenesis and Virulence Mechanisms**
(Linda Kenney / Eduardo A. Groisman / Vanessa Sperandio / Lori Burrows / Liz Sockett)



SPIROCHETES, BIOLOGY OF

Jan 31 - Feb 5, 2010

Ventura Beach Marriott
Ventura, CA

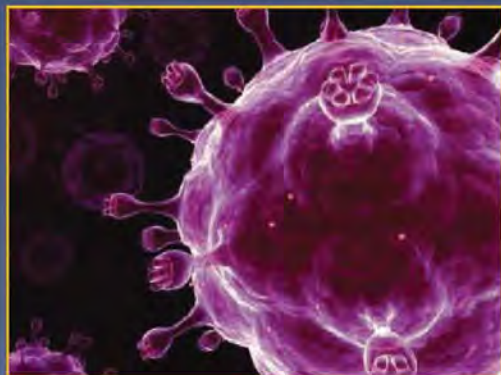
Chair: Richard P. Ellen

Vice Chair: Patricia A. Rosa

- **Advances in Genetics, Functional Genomics, Proteomics**
(Steve Norris / Lora Hooper / Ben Adler / Peter Uetz)
- **Gene Expression; Spirochetal Protein Structure and Function**
(Scott Samuels / Melissa Caimano / Mathieu Picardeau / Michael Norgard / Wolfram Zuckert)
- **Theoretical Biology Forum: Conceptual Leaps Forward, Spirochetal Research in the 21st Century**
(Sheila Lukehart / Linda Bockenstedt / Justin Radolf / Caroline Cameron / David Haake)
- **Biology Related to Risk for Infection**
(Jennifer Coburn / Yasmine Belkaid / Fang-Ting Liang / Chris Fenno / Brian Stevenson)
- **Late Breaking Research Session**
(Jennifer Miller, M. Motaleb)
- **Invasion and Evasion Strategies of Pathogenic Spirochetes**
(Nyles Charon / Chunhau (Chris) Li / Monica Embers / Richard Zuerner)
- **Interaction with Host Cells, Immunopathology**
(Gary Wormser / Janis Weis / Linden Hu)
- **Molecular Diversity, Environment, and Ecology**
(Tom Schwan / Alan Barbour / Ana L.T.O. Nascimento / Thad Stanton / Utpal Pal)

Gordon Research Conferences: "Session I" 2010 Preliminary Programs (continued)

- **Environmental Signals, Membrane Transport, and Metabolism of Spirochetes**
(Frank Gherardini / Richard Marconi / Julie Boylan / Sven Bergström)



NEW! THIOL-BASED REDOX REGULATION & SIGNALING

May 9-14, 2010

Il Ciocco Hotel and Resort

Lucca (Barga), Italy

Chair: Roberto Sitia

Vice Chair: Leslie B. Poole

- **Thiol-Regulated Molecular Machines**
(Michel Toledano / Vadim Gladyshev / Neil Hogg)
- **Redox Homeostasis and Signaling in the Cytosol**
(Arne Holmgren / John Mieyal / Elias Arner / Sue Goo Rhee / Christine Winterbourn)
- **Redox-Based Interorganellar Circuits**
(Chris Chang / Michel Toledano / Rafael Radi / Enrico Avvedimento)
- **Disulfide Bond Formation and Isomerisation in the ER and Mitochondria**
(Ari Helenius / Jonathan Weissman / Lars Ellgaard / Kaz Nagata / Agnes Chacinska)

- **Physiology of Redox Circuits**
(Sue Goo Rhee / David Ron / Costas Koumenis / Jim Imlay)
- **Pathology**
(David Ron / Ari Helenius / Hidde Ploegh)
- **Disease**
(Pier Giuseppe Pelicci / Pier Giuseppe Pelicci / Paola Chiarugi / C. Furdul)
- **Systems Biology of Redox Circuits**
(Jonathan Weissman / Elizabeth Veal / ChihCheh Wang)
- **Novel Tools**
(Chris Chang / Feroz Papa)

NEW! ULTRAFAST PHENOMENA IN COOPERATIVE SYSTEMS

Feb 28 - Mar 5, 2010

Hotel Galvez

Galveston, TX

Chair: Tony F. Heinz

Vice Chair: Alfred Leitenstorfer

- **Ultrafast Magnetism and Spin Dynamics**
(Paul van Loosdrecht / Theo Rasing / Joseph Orenstein)
- **Dynamics in Correlated Electron Materials**
(Jure Demsar / Antoinette Taylor)
- **Electronically Driven Phase Transitions**
(Nuh Gedik / Rupert Huber)
- **Phase Transitions Induced by Nonequilibrium Phonons**
(Robert Schoenlein / Andrea Cavalleri)
- **Ultrafast Electric Field-Induced Effects**
(Ulrich Hofer / Aaron Lindenberg)
- **Exciton and Polaron Dynamics**
(Mischa Bonn / Xiaoyang Zhu)
- **Attosecond Electron Dynamics in Solids**
(Alfred Leitenstorfer / Adrian Cavalieri)

- **Ultrafast Dynamics at Interfaces and in Nanoscale Systems**
(Hrvoje Petek / Martin Wolf / Junichiro Kono)
- **Young Investigator Presentations**
(Roberto Merlin)

VISUAL SYSTEM DEVELOPMENT

May 23-28, 2010

Il Ciocco Hotel and Resort

Lucca (Barga), Italy

Chair: Monica L. Vetter

Vice Chair: Helen McNeill

- **Frontiers in Visual System Research**
(Robin Ali / Dennis O'Leary)
- **Stem Cells, Progenitors and Proliferation**
(Tom Reh / Nick Baker / Michel Cayouette / Bill Harris)
- **Early Eye Development and Evolution**
(Francesca Pignoni / Michael Zuber / Justin Kumar / Julian Partridge / Dan Nilsson)
- **Development of Ocular Tissues**
(Richard Lang / Tiffany Cook / Ross Cagan / Holger Gerhardt)
- **Gene Regulation and Eye Development**
(Graeme Mardon / Richard Carthew / Ilaria Rebay / Ales Cvekl)
- **Cell Signaling and Morphogenesis**
(Valerie Wallace / Marek Mlodzik / Sabine Fuhrmann)
- **Development of Photosensitive Cells**
(Pamela Raymond / Claude Desplan / Ulrich Tepass / Steven Reppert / King-Wai Yau)
- **Connectivity in the Visual System**
(Iris Salecker / Christine Holt / Robin Hiesinger / Rachel Wong / Chi-Hon Lee)
- **Retinal Development and Disease**
(Tom Glaser / Mike Dyer / Utpal Banerjee / Kang Zhang)

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www.thermofisher.com



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The ESGRO Complete Plus medium is for feeder-free and serum-free culture of murine embryonic stem cells designed to maintain the cells' pluripotency. It includes the original ESGRO Complete clonal grade formulation, plus a glycogen synthase kinase 3 β (GSK3 β) inhibitor supplement to help prevent differentiation. Recent studies have shown the GSK3 β inhibitors help maintain pluripotency and self-renewal through the Wnt pathway. Cells cultured in the medium supplemented with the GSK3 β inhibitor consistently display better growth characteristics, cell morphology, viability, and proliferation rates compared with cells cultured in the original ESGRO Complete medium alone.

Millipore
For information 978-762-5170
www.millipore.com

Microvolume Spectrophotometer

The AlphaSpec is a unique in-tip microvolume ultraviolet/visible spectrophotometer. Its noncontact detection method allows as little as 2 μ l of sample to be measured while in a pipette tip, with complete recovery of the sample for downstream analysis. New analysis and measurement software enhances its workflow and performance. New features include automatic autoblanking; the ability to set up and store linear interpolation graphs based on known standards for Bradford, Lowry, or other common assays; and overlay of graphs to enable the comparison of replicate samples or to visualize changes in spectra at a glance. The new software enables one-click printing of all data and graphs, as well as the ability to export the data into Excel or ASCII format. The software can also display the microarray dye incorporation ratios of labeled oligonucleotides. It calculates both the concentration of each dye and the number of molecules per base.

Alpha Innotech
For information 800-795-5556
www.alphainnotech.com

Laboratory Information Management System

The Labworks LIMS version 6.1 software now supports Labworks WebTop for web client access. The WebTop requires no installation of software on the client device. Labworks users can run the program from a wide variety of web browsers, including wireless devices. The program can be deployed with minimal user training. In today's rapidly changing portable device market, it can protect users from hardware obsolescence. The software supports the unique needs of laboratories, from prelog-in sample organization through reporting

and data distribution. A configurable workflow allows laboratories to focus on only those process steps that are relevant to a sample lifecycle, allowing greater control over resources without changing the way the laboratory operates.

PerkinElmer
For information 781-663-6900
www.perkinelmer.com

Capillary Isoelectric Focusing

A new peptide-based, isoelectric point (pI) kit, the pI Marker Kit, is for the charge heterogeneity analysis of biotherapeutics based on advanced capillary isoelectric focusing techniques. The new synthetic pI calibrators attain high levels of precision in pI identification and isoform quantitation. They are packaged as a set containing markers for pI 4.1, 5.5, 6.7, 7.0, 9.5, and 10.0, each in sufficient quantity for 100 tests. The markers were developed for use on Beckman Coulter's PA 800 and PA 800 plus capillary electrophoresis systems.

Beckman Coulter
For information 714-993-8955
www.beckmancoulter.com

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The YC-1 Flow Chamber is designed for using time-lapse microscopy to monitor yeast cell growth in the presence of changing media. The unique flow cell of the YC-1 allows the user to seamlessly and rapidly change the liquid environment while observing yeast cell proliferation in a bidimensional manner over multiple cell cycles, all without cell washout. For use with inverted microscopes only, the YC-1 Flow Chamber offers models with resistive heating or Peltier temperature control options.

Warner Instruments
For information 800-599-4203
www.warnerinstruments.com

Flow Cytometry Kit

The Human/Mouse Embryonic Stem Cell 4-Color Flow Cytometry Kit contains four different fluorochrome-conjugated antibodies and corresponding isotype controls for single-step staining of human and mouse embryonic stem cells. The conjugated antibodies include anti-SOX2, anti-Oct-3/4, antiSSEA-1, and antiSSEA-4. The kit includes fixation, permeabilization, and wash buffers.

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Science Careers

From the journal *Science*



POSITIONS OPEN

OPEN FACULTY POSITIONS
Boston College Chemistry Department
Website: <http://www.bc.edu/chemistry>

The Chemistry Department at Boston College invites applications for two tenure-track positions with hire dates in the fall of 2010. Applicants will be evaluated on their potential to establish a prominent, externally funded research program and to excel in teaching both at the graduate and undergraduate levels. Successful applicants will join a Department of approximately 30 postdoctoral fellows, 125 doctoral students, 200 undergraduate chemistry majors, and an internationally recognized faculty. Individuals with expertise that complements other faculty in the Department are encouraged to apply.

ASSISTANT PROFESSOR OF CHEMICAL BIOLOGY (broadly defined): requires a Ph.D. in chemistry, biochemistry, or a related area and postdoctoral experience.

ASSISTANT PROFESSOR OF PHYSICAL CHEMISTRY (broadly defined, with the emphasis on materials chemistry): requires a Ph.D. in chemistry or a related area and postdoctoral experience.

Applicants must: (1) Submit a cover letter, curriculum vitae, summary of research plans, and statement of teaching philosophy (in one transmission using PDF files) to e-mail: chembiosearch@bc.edu or pchemsearch@bc.edu. Please specify chemical biology or physical chemistry search in your cover letter. (2) Arrange to have three letters of reference in PDF format transmitted to e-mail: chembiosearch@bc.edu or pchemsearch@bc.edu. Original letters may be requested by the Department. In your cover letter, please specify the names and contact information for your three references. (3) Submit all application materials electronically on or before 15 October 2009.

Boston College, a university of eight schools and colleges, is an Equal Opportunity Employer and supports Affirmative Action.

FACULTY POSITION Columbia University Department of Chemical Engineering

The Department of Chemical Engineering announces a faculty position to be filled at the rank of **ASSISTANT** or **ASSOCIATE PROFESSOR**. The Department seeks outstanding individuals with the motivation to excel in research, teaching, and service. Candidates at the Associate level should have a record of continued strong leadership in research. A Doctorate in chemical engineering or a related field is required. Departmental research is in biological, soft materials, electrochemical, or environmental engineering, and candidates that complement current departmental research will be given the highest priority. Columbia University offers an attractive, highly intellectual and collaborative environment. Assistance with faculty housing is available. The search will close no sooner than November 30, 2009, and will remain open until the position is filled. Starting date is July 1, 2010. Candidates should submit a brief research plan, statement of teaching objectives that demonstrates a commitment to chemical engineering education, the names and contact information of three references, curriculum vitae, and reprints of recent key research publications. Do not mail applications. Please apply online to website: <https://academicjobs.columbia.edu/applicants/Central?quickFind=51859>.

Columbia University is an Affirmative Action/Equal Opportunity Employer.

CAREER OPPORTUNITY

Doctor of Optometry (O.D.) degree in 27 months for Ph.D.s in science and M.D.s. Excellent career opportunities for O.D./Ph.D.s and O.D./M.D.s in research, education, industry, and clinical practice. This unique program starts in March of every year, featuring small classes and 12 months devoted to clinical care.

Contact the Admissions Office, telephone: 800-824-5526 at the New England College of Optometry, 424 Beacon Street, Boston, MA 02115. Additional information at website: <http://www.neco.edu>; e-mail: admissions@neco.edu.

POSITIONS OPEN



POSTDOCTORAL POSITION in Structural Biology University of Missouri

A Postdoctoral Position is available in nuclear magnetic resonance (NMR) structural biology. You should be a recent Ph.D. awardee with training in biomolecular NMR and expertise in quantitative analysis and biophysics. You will be involved in developing novel paramagnetic NMR techniques to visualize protein dynamics (*Nature* 444:383-386, 2006; 449: 1078-1082, 2007; and 455:693-696, 2008). Potential projects are (1) to visualize early events of retroviral capsid assembly and (2) to characterize domain movement of glutamate receptors. Please send your curriculum vitae and names and contact information of three references to: **Dr. Chun Tang, 117 Schweitzer Hall, Department of Biochemistry, University of Missouri, Columbia, MO 65211.** E-mail: tangch@missouri.edu.

THE ENDOWMENT FOR SCHOLARS IN BIOMEDICAL RESEARCH at The University of Texas Southwestern Medical Center

University of Texas Southwestern Medical Center is pleased to announce the continuation of the Endowed Program for Scholars in Biomedical Research. The Program, which is fully funded from private endowment, will provide at least \$1 million over four years to support the research activities of each new **ASSISTANT PROFESSOR** (tenure track) appointed to the Program. Academic appointments and research space will be provided by individual medical school departments or research centers. Positions in both basic science and clinical departments are available. The goal of the program is to assure a successful beginning of the research careers of an ever-growing cadre of outstanding and creative investigators at UT Southwestern.

For detailed information about currently available faculty positions, please access our website: <http://www8.utswmed.edu/utsw/home/scholars/index.html>.

SOUTHWESTERN The University of Texas Southwestern Medical Center At Dallas

UT Southwestern is an Equal Opportunity Institution.

MOLECULAR CARDIOLOGIST: ENDOWED CHAIR. The Molecular Cardiology Program at Weill Medical College of Cornell University seeks independent investigators as tenure-track/tenured faculty members. This position includes an endowed chair at **ASSOCIATE PROFESSOR** rank. The Molecular Cardiology Program is a vibrant multidisciplinary group with diverse interests including cardiovascular genetics, cardiovascular development, vascular biology, stem cell biology, signal transduction, and cellular electrophysiology. Successful candidates (M.D., M.D.-Ph.D., or Ph.D.) should have an established track record of extramural funding and will be provided with renovated research space and a generous startup package. Salary and rank will be commensurate with experience. Applicants should forward curriculum vitae, research plan, and three references to: **Ann Grgas, Assistant to the Molecular Cardiology Search Committee, Division of Cardiology, Cornell Medical College, Starr 4, 525 East 68th Street, New York, NY 10021.** Telephone: 212-746-2169; fax: 212-746-6951; e-mail: ang2010@med.cornell.edu. *Cornell is an Equal Opportunity Employer.*

HOWARD HUGHES MEDICAL INSTITUTE

Laboratory Head Positions at Janelia Farm

We invite applications from biochemists, biologists, chemists, computer scientists, engineers, mathematicians, neurobiologists, and physicists at all career stages who are passionate in their pursuit of important problems in basic scientific and technical research.

Appointments may be made at either of two levels:

Fellows

Fellows are independent scientists with labs of up to two additional members.

Appointments are for five years.

Group Leaders

Group leaders are independent scientists, similar to HHMI investigators, with labs of up to six additional members. The initial appointment is for six years. Thereafter, group leaders will be reviewed for reappointment every five years.

There are two application deadlines per year and the next are:

December 15, 2009 and July 15, 2010.

For more information and to submit an application:

www.hhmi.org/ref/janelia/sci

HHMI

HOWARD HUGHES MEDICAL INSTITUTE

At Janelia Farm, we pursue challenging basic biomedical problems for which future progress requires technological innovation. We focus on two research areas: the identification of general principles that govern how information is processed by neuronal circuits; and the development of imaging technologies and computational methods for image analysis. This year we have decided to broaden our foci at the Fellow level – we also seek very promising, early career stage scientists with interests beyond these two major foci. We expect that Janelia would be attractive to people with scientific programs that could benefit from collaborators or technologies already at Janelia. We value people with new perspectives who will contribute to our intellectual community.

Examples might include:

- ❖ A cell biologist looking to apply super-resolution optical microscopy to their work.
- ❖ A computer scientist interested in machine vision.
- ❖ A physicist interested in instrument development.
- ❖ A biochemist interested in single-molecule imaging.

Janelia Farm is now home to a growing, multidisciplinary community of 35 research groups, comprising postdoctoral associates, graduate students, and technicians. Our scientists are supported by outstanding shared resource facilities within a unique campus less than an hour from Washington, D.C. All laboratories are internally funded, without extramural grants. Lab heads have no formal teaching duties and minimal administrative responsibilities. Janelia Farm offers a supportive working environment with on-site child care, fitness center, and dining facilities.

Individual research groups are limited in size. We value research collaboration between groups as a mechanism to enable long-range innovative science and encourage the self-assembly of interdisciplinary teams of scientists. In addition we support external collaborative science through a scientific visitor program.

The Howard Hughes Medical Institute is an equal opportunity employer. Women and members of racial and ethnic groups traditionally underrepresented in the biomedical sciences are encouraged to apply.

Director of the Environmental and Occupational Health Sciences Institute (EOHSI)

UMDNJ-Robert Wood Johnson Medical School (RWJMS) and
Rutgers University
and

Chair of the Department of Environmental and Occupational Medicine (DEOM)

UMDNJ-Robert Wood Johnson Medical School
Piscataway, New Jersey

We are seeking candidates for the position of Director of EOHSI, a jointly sponsored institute of UMDNJ-RWJMS and Rutgers University, and Chair of DEOM, UMDNJ-RWJMS.

The ideal candidate would assume both positions and must have a PhD or equivalent or MD (New Jersey licensure or eligible). The candidate must have a distinguished record of research and/or clinical service, as well as teaching and be able to lead and collaborate within a highly productive and collegial multidisciplinary environment, develop programs, and raise funds to further EOHSI's mission. The successful candidate must articulate a clear vision of research, demonstrated mentoring skills and service to government and the public, as well as a commitment to fostering interactions with other academic units.

EOHSI's mission encompasses basic and clinical research in the environmental health sciences, environmental education and public policy. EOHSI serves as an unbiased source of expertise on environmental problems. The Institute's four-story, 78,000-square-foot building opened in 1991. It houses an NIEHS Center for Environmental Health Sciences, Environmental and Occupational Health Clinic, an Ozone Research Center, an EPA Environmental Bioinformatics and Computational Toxicology Center, a Computational Chemodynamics Laboratory, a CounterAct Center and a Controlled Environment Facility for stimulating human exposure to pollutants. EOHSI is home to three graduate programs: The Joint Graduate Program in Toxicology, The Joint Doctoral Program in Exposure Assessment, and the Residency Program in Occupational and Environmental Medicine. For further details go to eohsi.rutgers.edu. The DEOM is part of UMDNJ-RWJMS, and its research, academic and clinical programs are closely linked with EOHSI.

Applicants should apply in confidence to **Dr. Bonnie Baloga-Altieri** at balogabl@umdnj.edu or UMDNJ-RWJMS, Clinical Academic Building, Suite 1400, 125 Paterson Street, New Brunswick, NJ 08903. Your application should include a letter stating qualifications for the position, current curriculum vitae, and the names and contact information of three professional references. Review of applications will be ongoing process until the position is filled. The University of Medicine and Dentistry of New Jersey and Rutgers University are both affirmative action and equal opportunity employers. For more information, visit www.umdnj.edu/hrweb and www.rutgers.edu.



ROBERT WOOD JOHNSON
MEDICAL SCHOOL
University of Medicine & Dentistry of New Jersey

RUTGERS
STATE UNIVERSITY OF NEW JERSEY



Washington University in St. Louis

SCHOOL OF MEDICINE

Faculty Position in Chemical Biology

The DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR BIOPHYSICS at Washington University School of Medicine invites applications for tenure-track and tenured faculty positions from candidates using synthetic chemistry as a tool to probe biological systems. Compatible areas of research include the application of synthetic chemistry methodologies and insights to understanding the regulation of biochemical pathways and cellular physiology, the discovery of new therapeutic targets, and the study of basic mechanisms of biomolecular recognition, catalysis, and protein function. The successful candidate will combine synthetic chemistry methodologies with a strong program in basic biological research. She/he will join a growing department that is broadly represented in experimental and computational studies of protein physical chemistry, structure, and mechanistic enzymology. A state-of-the-art synthetic chemistry facility is under construction that will support and integrate small molecule chemistry with biochemical investigations of macromolecules (<http://www.biochem.wustl.edu>). The targeted growth of systems biology, nanomaterials research, and biological chemistry on both campuses of Washington University will provide additional opportunities for scientific interactions and collaborations. In addition to a commitment to research, a candidate's enthusiasm for teaching and mentoring young scientists is also important. Applicants at the Assistant, Associate, or Full Professor level will be considered.

Washington University has a highly interactive research environment with vigorous interdisciplinary graduate and medical scientist training programs. Applicants should submit their curriculum vitae, selected reprints, a short summary of future research plans and the names of references electronically to faculty-search@biochem.wustl.edu, or else by mail to: **CHEMICAL BIOLOGY SEARCH**, Tom Ellenberger, Raymond H. Wittcoff Professor and Head, Department of Biochemistry and Molecular Biophysics, Box 8231, Washington University School of Medicine, 660 S. Euclid Ave., St. Louis, MO 63110. Applications will be reviewed beginning in October, 2009 and the search will be closed on January 15, 2010.

AN EQUAL OPPORTUNITY EMPLOYER.

Minority and women scientists are especially encouraged to apply.



Washington University in St. Louis

SCHOOL OF MEDICINE

Faculty Position in Structural Biology

The DEPARTMENT OF BIOCHEMISTRY AND MOLECULAR BIOPHYSICS at Washington University School of Medicine invites applications for a tenure-track or tenured faculty position in the area of Structural Biology. Applicants at the Assistant, Associate, or Full Professor level will be considered. The successful candidate will conduct independent research within an active, growing department broadly interested in quantitative studies of macromolecular interactions, mechanistic enzymology, molecular dynamics and structure/function relationships (<http://biochem.wustl.edu>). Priority will be given to multidisciplinary approaches to study biological function(s). Enthusiasm for teaching and mentoring research trainees is important.

Washington University has a highly interactive research environment with vigorous interdisciplinary graduate and medical scientist training programs. Selection of candidates will begin in November 2009. Applicants should submit their curriculum vitae, selected reprints, a short summary of future research plans and the names of references electronically to: structure-search@biochem.wustl.edu or else by mail to:

STRUCTURAL BIOLOGY SEARCH

Tom Ellenberger, Raymond H. Wittcoff Professor and Head
Department of Biochemistry and Molecular Biophysics
Washington University School of Medicine
660 S. Euclid Ave., Box 8231
St. Louis, MO 63110

AN EQUAL OPPORTUNITY EMPLOYER.

Minority and women scientists are especially encouraged to apply.

INRA (French National Institute for Agricultural Research) seeks project proposals for four "scientific packages".

This call for proposals is open to experienced researchers specialising in INRA's priority research areas. The purpose is to allow a laboratory (research unit) of the Institute to welcome a senior researcher and provide strong support for a site or a key scientific undertaking. Awardees will be offered generous means, in terms of salary and scientific facilities, for a four-year period. Deadline for the submission of applications is set for 15 October 2009. Information on the research unit ready to welcome the applicant must be filled in the application form.

Download the call for proposals and the application forms:

www.international.inra.fr/join_us

Contact: package@paris.inra.fr

orc.fr



Center for
stem cell biology

at the University of Michigan

The University of Michigan invites applications for tenure track **ASSISTANT PROFESSOR** positions. We are seeking outstanding scholars, with Ph.D., M.D. or equivalent degrees and relevant postdoctoral experience, who show exceptional potential to develop an independent research program that will address fundamental issues in any aspect of stem cell biology. Applicants who have already established successful independent research programs will be considered for tenured **ASSOCIATE PROFESSOR** or **PROFESSOR** positions.

Applicants should send a curriculum vitae, copies of up to three reprints, a one- to two-page summary of research plans, and arrange to have three letters of reference sent directly by **October 31, 2009** to: **Stem Cell Search Committee, c/o Rebecca Fritts, Life Sciences Institute, University of Michigan, 210 Washtenaw Avenue, Ann Arbor, Michigan, 48109-2216.**

The University of Michigan is an Affirmative Action/Equal Opportunity Employer.

King Abdullah University of
Science and Technology



DEAN, CHEMICAL AND LIFE SCIENCES & ENGINEERING and DEAN, PHYSICAL SCIENCES & ENGINEERING

King Abdullah University of Science and Technology (KAUST) is a new private international graduate research university governed by an independent, self-perpetuating Board of Trustees. Integrating education and research, KAUST been generously endowed to support the educational and research activities of the faculty and students. The merit-based university, which employs the highest international standards of scholarship, research, education, and learning, is open to all without regard to race, color, religion, or gender. Opening its doors to students in September 2009, KAUST's pioneer class of 400 students, 25% of whom are women, comes from 60 countries. At maturity, KAUST will enroll 2,000 students in MS and PhD programs. The medium of instruction is English. The environmentally friendly campus with state-of-the-art facilities is built on 36 square kilometers on the shore of the Red Sea near Jeddah, Saudi Arabia's second largest city.

KAUST provides an open intellectual environment for its faculty and students. To this end, KAUST is organized around a matrix structure of Academic Divisions and interdisciplinary Research Centers. There are three Academic Divisions, each directed by a Dean. The Divisions and their degree programs are broadly defined to encourage interdisciplinary activity.

Academic Divisions

Chemical and Life Sciences & Engineering

Mathematical and Computer Sciences & Engineering (**Dr. David Keyes, Dean**)

Physical Sciences & Engineering

Degree Programs

Bioscience
Chemical and Biological Engineering
Chemical Science
Environmental Science & Engineering
Marine Science & Engineering

Applied Mathematics and Computational Science
Computer Science

Electrical Engineering
Materials Science & Engineering
Mechanical Engineering
Earth Science & Engineering

The Deans are expected to raise the visibility and standing of KAUST through building educational and research programs of the highest quality and leveraging collaborative and interdisciplinary activities across Divisions. They are responsible for the educational and degree-granting programs, and baseline research funding for their divisions, including:

- faculty administration and budget preparation;
- faculty recruitment, evaluation, promotion, compensation; and
- degree requirements and teaching assignments.

Detailed information on Academic Divisions, Research Centers, and the Global Collaborative Research program encompassing partnerships around the world can be found on the KAUST website (www.kaust.edu.sa).

Contact Information: Korn/Ferry International, which is assisting with this search, invites confidential inquiries, nominations and applications. All communications will be held in absolute confidence. Nominations should include nominee contact information. Applications should include a CV and letter explaining interest and relevant experience. The positions are available during academic year 2009-10.

Korn/Ferry International

John Kuhnle, Managing Director-Global Education Practice

Elizabeth Dycus, Senior Associate

802/765-4543

KAUSTchemical@kornferry.com

KAUSTphysical@kornferry.com



Faculty Positions in Burnett School of Biomedical Sciences College of Medicine

**Cancer, Cardiovascular and Metabolic Diseases,
Infectious Diseases and Neurodegenerative Diseases**



University of Central Florida is expanding its Biomedical Research and Education Program into a new 198,000sq.ft. Burnett Biomedical Science building in the new medical campus. We seek outstanding scientists working on molecular, cellular, physiological, biochemical, or pharmacological approaches to study important problems with relevance to cancer, cardiovascular and metabolic diseases, infectious diseases and neurodegenerative diseases. Faculty at Assistant, Associate or Full Professor level will be considered. Successful applicants will be expected to establish a well funded research program, contribute to teaching, and actively participate in MS and PhD programs.

Competitive salaries, startup funds, new laboratories, transgenic animal facilities and access to shared core instrumentation facilities will be provided. Burnett School researchers will have access to the extensive core facilities in the adjacent Burnham Medical Research Institute. Medical campus is part of multibillion dollar biomedical cluster that will include the new Medical School, Burnham Medical Research Institute, Veterans Administration Hospital and Nemours Children's Hospital.

The University of Central Florida has over 50,000 students and an outstanding technology-based infrastructure. It is located in Orlando, a dynamic and progressive metropolitan region, a major player in high-tech industry, and adjacent to a top ranked Research park and a great place to live and work.

Review of candidates will begin on **October 15, 2009**. Please apply to the links below by submitting a curriculum vitae, a two page summary of research plans and contact information for three or more references.

- **Cancer:** www.jobswithucf.com/applicants/Central?quickFind=74185
- **Cardiovascular and Metabolic Diseases:**
www.jobswithucf.com/applicants/Central?quickFind=74186
- **Infectious Disease:** www.jobswithucf.com/applicants/Central?quickFind=74188
- **Neurodegenerative Disease:** www.jobswithucf.com/applicants/Central?quickFind=74189

The University of Central Florida is an Equal Opportunity, Equal Access, and Affirmative Action Employer. As a member of the Florida State University System, all application materials and selection procedures are available for public review.



BROAD FELLOWS PROGRAM IN BRAIN CIRCUITRY CALIFORNIA INSTITUTE OF TECHNOLOGY

THE CALIFORNIA INSTITUTE OF TECHNOLOGY is looking for a few outstanding scientists from any relevant backgrounds to study how networks of neurons give rise to perception, memory, emotion, and behavior. We encourage applications from individuals employing genetic manipulations in relevant animal model systems, electrophysiological recordings, functional imaging and computational analyses and related tools. Broad Fellows are independent researchers who have recently received their PhD. They will receive internal funding for a group of up to three people (up to \$150,000 in direct costs per year per fellow). The initial appointment is for three years, with the possibility of renewal for one more year. Excellent salary (\$75,000 per year) and benefits. Applications should include curriculum vitae, a statement of research plan, and three letters of recommendation. This material should be submitted online at: <http://www.broadfellows.caltech.edu> or by email to heather@klab.caltech.edu by **November 15th, 2009**.

Caltech is an Equal Opportunity/Affirmative Action Employer. Women, minorities, veterans, and disabled persons are encouraged to apply.



the foundations



The University of Neuchâtel, Switzerland, offers the position of:

A Full professor (professeur ordinaire) in Biology of arthropod vectors of pathogens

The candidate is expected to have a strong research background which complements well with the parasitology interests of the Institute. He/she will have an experience in the biology of vectors of pathogens focused on the physiology of ectoparasites, capable to combine both field and laboratory methods and showing an international recognized research program. He/she is asked to teach comparative animal physiology, histology and cytology at the bachelor level, plus specialized courses in his/her research field of the master in Biology of Parasites and Ecoethology.

Full chair (6 hours weekly teaching in French and English, research activities as well as administrative tasks).

Starting date: February 1, 2010 or date to be discussed.

The University of Neuchâtel encourages women to apply.

Candidates with a Ph.D. should submit their application, both electronically and on paper, **15 October 2009** to Prof. Bruno Betschart, head of the search committee, Institute of Biology, Faculty of Science, University of Neuchâtel, PO Box 158, 2009 Neuchâtel - Switzerland, email: bruno.betschart@unine.ch.

Applications should include curriculum vitae with a description of research, teaching, grants, and administrative records; a complete list of publications; a copy of academic diplomas; and a research program (describing the candidate's scientific vision and the projects to be developed at the University of Neuchâtel) of up to 3 pages. The candidate should ask three experts to send a letter of recommendation to the head of the search committee. Further information is available from Prof. B. Betschart or on the web site www.unine.ch/sciences, under « emploi ».

Faculty Position Molecular Biology Sloan-Kettering Institute

The Molecular Biology Program of the Sloan-Kettering Institute, Memorial Sloan-Kettering Cancer Center (www.ski.edu), has initiated a faculty search at the Assistant Member level (equivalent to Assistant Professor). We are interested in outstanding individuals who have demonstrated records of significant accomplishment and the potential to make substantial contributions to the biological sciences as independent investigators. Successful applicants will have research interests that move the Program into exciting new areas that complement and expand our existing strengths in the areas of maintenance of genomic integrity, regulation of the cell cycle, and regulation of gene expression. Faculty will be eligible to hold appointments in the Gerstner Sloan-Kettering Graduate School of Biomedical Sciences, the Weill Cornell Graduate School of Medical Sciences, as well as the Tri-Institutional MD/PhD Training Program.

Candidates should e-mail their application in PDF format to: molbio@mskcc.org by **November 16, 2009**. The application should include a CV, description of past and proposed research (3-7 pp), and copies of three representative publications. Candidates should arrange to have three signed letters of reference sent in PDF format to: molbio@mskcc.org. The letters should arrive by **November 16, 2009** and should be addressed to **Dr. Kenneth Mariani, c/o Julie Kwan, Box 135, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, New York 10065**. Inquiries may be sent to Ms. Kwan at molbio@mskcc.org or to Dr. Kenneth Mariani, Chair, Molecular Biology Program at kmarians@sloan-kettering.edu. Memorial Sloan-Kettering Cancer Center is an Equal Opportunity Employer and smoke-free environment.



**Memorial Sloan-Kettering
Cancer Center**

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From technology specialists to patent attorneys to policy advisers, learn more about the types of careers that scientists can pursue and the skills needed in order to succeed in nonresearch careers.

Science Careers

From the journal *Science* AAAS



WE MAKE LIVES BETTER

UT HEALTH SCIENCE CENTER™
SAN ANTONIO

San Antonio, Texas

Dean, Graduate School of Biomedical Sciences

The University of Texas Health Science Center at San Antonio is searching for a dynamic leader who will assume the position of Dean of the Graduate School of Biomedical Sciences, one of the five Schools at the UT Health Science Center. The UT Health Science Center is in the top 2% of all research universities in the nation receiving federal funding and is the largest research intensive university in South Texas. The School serves as the chief catalyst for the \$16.3 billion biosciences and healthcare industry in San Antonio. This individual will have responsibility for resources and direction of the Graduate School, which includes seven departments with 147 tenure track faculty and 354 MS and PhD students. The position reports to the President of the University. This individual will control resources and take primary responsibility for planning the future direction and expansion of the Graduate School, which has current extramural funding in excess of \$40 million. Total extramural funding at the UT Health Science Center is now \$202 million. A dramatic increase in research space of more than 225,000 square feet and significant funding increases make this a unique opportunity for a visionary leader.

The successful candidate will hold a PhD, MD/PhD or DDS/PhD and be an outstanding scientist and scholar with an international reputation. He/she should have a strong record of accomplishment in research, education and administration. In addition, this individual should have a track record of success in recruiting high caliber individuals and developing collaborative faculty interactions.

This outstanding leader will have responsibilities to advance the quality of graduate education and research endeavors in the biomedical sciences and foster interdisciplinary graduate education and collaboration between the Graduate School of Biomedical Sciences and other partners. The successful candidate will also provide strong administrative leadership of graduate degree programs, admissions, student support programs, and postdoctoral scholars. He/she will have responsibility to review programs currently in place and be a bridge builder who understands and embraces accountability and stewardship of graduate school resources. He/she will work with the leadership of four other professional schools (Medical, Dental, Nursing, Health Professions) in creating and successfully implementing programs to enhance graduate student and postdoctoral scholar life and improve academic enrichment and translational science programs. In addition, the successful candidate will oversee a variety of outreach and diversity programs aimed to increase the participation of a diverse group in the graduate health sciences.

Nominations and CVs may be sent to the Health Science Center's search consultant, Marvene Eastham, at UTBioMed@wittkieffer.com. Items that cannot be emailed may be sent to 10375 Richmond Avenue, Suite 1625, Houston, TX 77042. We may be reached confidentially at 713-266-6779 (p) or 713-266-8133 (f). The UT Health Science Center is an Equal Employment Opportunity/ Affirmative Action Employer. All faculty and Executive Committee appointments are designated as security sensitive positions.

WITT / KIEFFER



United States Department of State Washington, DC

The United States Department of State invites applications for visiting scholar positions during the 2010-2011 academic years, per Section 202 of the Arms Control and Disarmament Act, as amended (22 U.S.C 2568).

Positions of **Physical Scientist, Biological Scientist, Chemical Scientist, and Political Scientist** are available at the Department of State in 2010-2011. The candidates selected under this program will have an opportunity for active participation in the arms control, nonproliferation, and disarmament activities of the Department of State and to enable the Department to gain the perspective and expertise such persons can offer.

The Department of State reimburses institutions of Foster Fellows for their salary and benefits, and Foster fellows receive travel reimbursement and a per diem allowance (subject to some restrictions).

For complete information about the program and positions, see <http://www.state.gov/t/>. To apply, please send a letter describing your perspective and expertise, a CV, three letters of reference, and two published articles to: **Thomas J. Yehl, VCI/TA, U.S. Dept. of State, Washington, DC 20520**. Receipt deadline is **September 30, 2009**.

Applicants must be U.S. citizens and will be subject to a security background investigation in the event of their selection for the program. Contact **Ms. Annette Day** at **202-647-4153** for more detailed information.

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Science Careers

From the journal *Science*

Faculty Position Cell Biology Program Sloan-Kettering Institute

The Cell Biology Program, Sloan-Kettering Institute (www.ski.edu) has initiated a search for tenure-track faculty members. We are interested in outstanding individuals who have the potential to develop an innovative, independent research program that complements and enhances our existing strengths. Candidates with research interests in exciting areas of eukaryotic cell biology and using a variety of experimental approaches and systems are encouraged to apply. New faculty will be eligible to hold graduate school appointments in the Gerstner Sloan-Kettering Graduate School of Biomedical Sciences, the Weill Graduate School of Medical Sciences of Cornell University, as well as the Tri-Institutional MD/PhD Training Program. Sloan-Kettering has an outstanding infrastructure as well as state-of-the-art core resources, and we are now significantly expanding our research programs.

Interested individuals should e-mail their application, preferably in PDF format, to Alan Hall PhD, Chair Cell Biology Program at: cellbio@mskcc.org by November 1st, 2009. The application should include a Curriculum Vitae, a description of past research accomplishments and proposed future research (3-7 pages) and three representative publications. Candidates should also arrange to have three signed letters of recommendation on letterhead in PDF format sent by e-mail to: cellbio@mskcc.org. The letters should arrive by November 1st, 2009. Inquiries can also be made by e-mail to: cellbio@mskcc.org. Memorial Sloan-Kettering Cancer Center is an affirmative action, equal opportunity employer.



Memorial Sloan-Kettering
Cancer Center
www.mskcc.org



The Nanoscience Cooperative Research Center CIC nanoGUNE Consolider (www.nanogune.eu) invites applications and nominations for the position of

Scientific Director

of a new start-up company that is being launched as a joint venture of private investors and CIC nanoGUNE Consolider. The company is located in San Sebastian, Basque Country (Spain), with the mission of developing graphene-based process and product technology as well as conducting related research activities.

The Scientific Director will be responsible for the design and management of the company's R&D strategy, the build-up and operation of its research laboratory and team, which will be based at the nanoGUNE facilities, as well as the development of a high-impact IP portfolio. The Scientific Director will also manage the coordination of the company's activities, which will utilize nanoGUNE's state-of-the-art research infrastructure for nanoscience and nanotechnology.

Candidates should have an outstanding track record in research and technology or process development, with a preferred expertise in the field of thin-film or nano-materials growth and related areas of surface science, the ability to lead and manage a research team and build up a world class research operation, as well as substantial experience with IP related issues. Proficiency in spoken and written English is compulsory; knowledge of Spanish is not a requirement.

Applicants should forward their CV and a list of at least three references to director@nanogune.eu

Closing date: **30 September 2009**



MAX PLANCK FLORIDA INSTITUTE

**2 Max Planck Research Group
Leader positions
(Associate Professor level)**

The newly established Max Planck Florida Institute, located on the FAU campus in Jupiter, FL, near Scripps Florida will focus its research on bioimaging at the cellular and molecular level. Nobel Laureate Prof. Dr. Bert Sakmann will serve as the inaugural scientific director. Applications for the following research areas are invited:

- Single molecule imaging
- Imaging of synapse dynamics
- Dense EM reconstruction of neural circuits
- Optogenetics and behavior

Candidates are expected to have a record of outstanding and internationally recognized research accomplishments in the above areas.

The positions are funded for a period of initially 5 years, with the possibility of two extensions 2 years each. Each position includes a start-up grant for equipment and salaries for one post-doc, two graduate students and one technician, as well as operating funds.

Application & details available at maxplanckflorida.org
Additional questions to Dr. Claudia Hillinger, VP for Institute Development: claudia.hillinger@maxplanckflorida.org

Deadline for Applications: September 25, 2009

The Max Planck Florida Institute is an Equal Opportunity Employer.



UNIVERSITY OF UTAH SCHOOL OF MEDICINE

A tenure track appointment is available for autumn 2010. The general areas of interest include, among other possibilities, protein and nucleic acids biochemistry, cellular function, metabolism, enzymology, signal transduction, and computational analysis. Applicants should hold a doctoral degree and have a strong record of research accomplishment. Preference will be given to applicants at the level of Assistant Professor. The successful candidate will join an interactive and diverse faculty, and will participate in campus-wide graduate training programs. Send curriculum vitae, statements of research background and future interest, and have three letters of recommendation sent to:

**Faculty Search Committee
Department of Biochemistry
University of Utah School of Medicine
15 N Medical Dr. East, Rm 4100
Salt Lake City UT 84112-5650 USA**

Or send via email to: BiochemSearch@lists.utah.edu

The University of Utah values candidates who have experience working in settings with students from diverse backgrounds, and possess a demonstrated commitment to improving access to higher education for historically under-represented students.

Deadline for applications is **November 15, 2009**, late applications will be considered if an opening is still available.

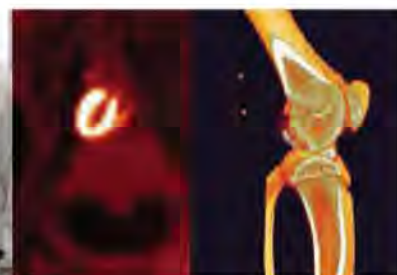
The University of Utah is an Affirmative Action/Equal Opportunity Employer and does not discriminate based upon race, national origin, color, religion, sex, age, sexual orientation, gender identity/ expression, disability, or status as a Protected Veteran. Upon request, reasonable accommodations in the application process will be provided to individuals with disabilities. To inquire about the University's nondiscrimination policy or to request disability accommodation, please contact: Director, Office of Equal Opportunity and Affirmative Action, 201 S. Presidents Circle, Rm 135, (801)581-8365.

GRADUATE PROGRAM

EBERHARD-KARLS
UNIVERSITÄT
TÜBINGEN



Laboratory for Preclinical
Imaging and Imaging Technology
of the Werner Siemens-Foundation



PhD Research Training Group Preclinical Molecular Imaging

Department of Radiology · University of Tübingen

OUR VISION IS...

...to encourage graduate students to take up training in the very exciting, innovative and interdisciplinary research field of "preclinical molecular imaging", an emerging research tool, which has an impact on various biomedical research areas such as neurology, oncology, cardiology and immunology.

The curriculum includes:

- > A rich program of courses and lectures
- > Workshops
- > Off-site meetings
- > Participation at conferences
- > Awards
- > An own research budget for every student
- > First-class guest speakers from internationally reputable laboratories
- > Optional research stay abroad (USA)

Who is eligible:

Graduate students should have a background in at least one of the following fields:

- > Biochemistry
- > Biology
- > Biophysics
- > Chemistry
- > Computer science
- > Medicine
- > Physics

APPLICATION DEADLINES:

**First Deadline 1st October 2009
Second Deadline 1st May 2010**

How to apply

Please visit our web-page to download the application forms and for further information.

www.preclinicalimaging.org

Contact

Prof. Dr. rer. nat. Bernd Pichler
Laboratory for Preclinical Imaging and Imaging Technology of
the Werner Siemens-Foundation
Department of Radiology
Eberhard-Karls-University Tübingen
Röntgenweg 13
72076 Tübingen, Germany
E-mail: bernd.pichler@med.uni-tuebingen.de
Phone: +49-7071-29-83427



INDIANA UNIVERSITY

Faculty Position Indiana University School of Medicine-South Bend Cell and Molecular Biology

The Indiana University School of Medicine-South Bend at the University of Notre Dame invites applications for a tenure-track faculty position in the area of cell and molecular biology at the Assistant or Associate Professor rank. Investigators with expertise in cancer biology and an emphasis in one or more of the following disciplines are particularly encouraged to apply: neuroscience, cell signaling, nanotechnology, genomics, proteomics, infectious disease, and immunology. Applicants must demonstrate an exceptional record of research accomplishment based on publications, national recognition, and extramural funding. State-of-the-art research facilities will be available in the newly constructed Harper Cancer Institute, a collaborative research venture between IU School of Medicine and Notre Dame. The successful candidate will be expected to teach IU medical students and contribute to graduate courses at the University of Notre Dame. Ample opportunities exist for collaborations with multiple departments in the School of Medicine and Notre Dame, as well as with interdisciplinary Centers of Excellence, including the Walther Cancer Institute, the Eck Institute for Global Health, the W.M. Keck Center for Transgene Research, and the Center for the Study of Biocomplexity. Additional information on the faculty and facilities is available at <http://medicine.iu.edu/southbend> and <http://science.nd.edu>.

Qualified individuals should send their curriculum vitae, the names and addresses of three references, and a summary of current and future research and teaching interests to: **Rudolph M. Navari, Chair, Search Committee, Indiana University School of Medicine-South Bend, 1234 Notre Dame Avenue, South Bend, IN 46617** (navari.1@nd.edu).

Indiana University School of Medicine-South Bend is an Affirmative Action/Equal Opportunity Employer. Women and minority candidates are encouraged to apply.

Faculty Positions: Center for Cell Engineering Memorial Sloan-Kettering Cancer Center

The Center for Cell Engineering (CCE) at Memorial Sloan-Kettering Cancer Center is seeking innovative individuals, for tenure-track positions at the Assistant Member level, with strong research accomplishments in stem cell research, cell engineering and/or cell therapy. The CCE focuses on human stem cell biology and cell-based immunotherapies. Successful applicants will have access to outstanding resources, including state-of-the-art facilities for cGMP cell processing, cell purification, imaging, vector production, transgene monitoring and genomic analyses. Faculty will be eligible to hold appointments in the Gerstner Sloan-Kettering Graduate School of Biomedical Sciences, the Weill Cornell Graduate School of Medical Sciences, as well as the Tri-Institutional MD/PhD Training Program.

MSKCC offers a unique and exciting research environment with programs in: Pharmacology, Chemistry, Developmental Biology, Immunology, Molecular Biology, Computational Biology, Genetics, Cell Biology, Cancer Pathogenesis and Structural Biology. The presence on campus of world-renowned clinical programs in cancer research, treatment and prevention offers many opportunities for effective translational research.

Applicants should have an MD or PhD degree, productive postdoctoral experience, and dedication to important problems related to human cell engineering and the development of innovative cell therapies.

Candidates should e-mail their application, preferably in PDF format, to celleng@mskcc.org by November 15, 2009. The application should include a CV, a description of past and proposed research (3-7 pages), and copies of three representative publications. Candidates should arrange to have three signed letters of reference in PDF format sent by e-mail to celleng@mskcc.org. Please address to: Michel Sadelain, MD, PhD, Center for Cell Engineering, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, Box 182, New York, NY 10065. Memorial Sloan-Kettering Cancer Center is an affirmative action, equal opportunity employer.



Memorial Sloan-Kettering
Cancer Center

www.mskcc.org



Universität Karlsruhe (TH)
Forschungsuniversität • gegründet 1825



The Faculty of Chemistry and Biosciences at the University of Karlsruhe (TH) has an opening for a

W3-Professorship for Theoretical Biophysics

to be recruited at the earliest possible date within the framework of the excellence initiative.

The candidate is expected to carry out basic research in theoretical biophysics. These activities should complement the current research topics of the life sciences in Karlsruhe ("structure of biological interfaces", "biological functionality of nanostructures", "structure and dynamics of molecular interactions") and strengthen related interdisciplinary research activities within the Karlsruhe Institute of Technology (KIT). Research topics should be focussed on the field of cellular biophysics (e.g. modelling of cell-cell or cell-surface interactions, theoretical analysis of cellular mechanics or physics of tissues) or molecular biophysics (e.g. modelling of multiprotein complexes, biomembrane systems, protein networks or intracellular signalling cascades).

We expect candidates to teach biophysics and cell biology at the undergraduate and graduate level within the course programmes of Biology and Chemical Biology. Biophysics teaching for graduate students of Physics and Chemistry is also encouraged. The successful candidate is expected to contribute to administrative and curricular activities in the Faculty.

The candidate should have a Habilitation or equivalent scientific standing and experience. Applications from qualified women are strongly encouraged. Handicapped applicants will be treated preferentially if equally qualified.

Applications containing the usual supporting materials and including five selected reprints should be addressed by **October 15, 2009** to the **Dean of Faculty of Chemistry and Biosciences, University of Karlsruhe (TH), Kaiserstr. 12, 76131 Karlsruhe, Germany.**



CIFAR

Junior Fellowship in Integrated Microbial Biodiversity

The **Integrated Microbial Biodiversity Program** of the Canadian Institute for Advanced Research (CIFAR) is seeking an exceptional postdoctoral researcher to fill a two-year Junior Fellow position to begin as early as December 1, 2009. CIFAR is a catalyst for discovery, incubating ideas that revolutionize the international research community. The Integrated Microbial Biodiversity (IMB) Program is comprised of a leading group of scientists whose goal is to explore and understand the astounding diversity of bacteria, viruses and other microbes on our planet.

The focus of this position is on the evolution of associations between insects and protists. The Junior Fellow will work on highly collaborative research between the labs of **Steve Perlmán** (ecology and evolution of insects and their associates) at the University of Victoria, and **Patrick Keeling** (protist genomics and evolution) at the University of British Columbia. The applicant may use either lab as a home base (or both, one in each year). The project area is open but may include the following: (a) **Tripartite symbioses between termites, protists, and bacteria;** (b) **Evolution of Drosophila-protist associations;** (c) **Molecular approaches to the study of microsporidian male-killers.** The successful candidate will have an outstanding record of accomplishment in a related research area, excellent communication skills, and strong potential to collaborate with program members. During his or her tenure, the Junior Fellow will be integrated as a member of the IMB Program and will also participate in CIFAR's elite Junior Fellow Academy. For more information, please see the full advertisement posted at <http://www.cifar.ca/JFA>.

Applications must include a CV (including list of publications) and a brief (1-page) statement of research interests. Materials should be sent to stevep@uvic.ca or pkeeling@interchange.ubc.ca. Applicants should also arrange for three letters of reference, not from CIFAR members, to be sent to the same address. To receive full consideration, applications must be received by **October 9, 2009.**

UNIVERSITY OF MICHIGAN

SCHOLARS PROGRAMS

BIOLOGICAL SCIENCES SCHOLARS PROGRAM For Junior, Tenure-Track Faculty

The University of Michigan announces recruitment for the Biological Sciences Scholars Program (BSSP) to continue to enhance its investigational strengths in the life sciences research programs.

Now entering its 13th year, this Program has led to the recruitment of outstanding young scientists in the areas of genetics, microbiology, immunology, virology, structural biology, pharmacology, biochemistry, molecular pharmacology, stem cell biology, cancer biology, physiology, cell and developmental biology, and the neurosciences. The Program seeks individuals with PhD, MD, or MD/PhD degrees, at least two years of postdoctoral research experience, and evidence of superlative scientific accomplishment and scholarly promise. Successful candidates will be expected to establish a vigorous, externally-funded research program, and to become leaders in departmental and program activities, including teaching at the medical, graduate, and/or undergraduate levels. Primary college and department affiliation will be determined by the applicant's qualifications and by relevance of the applicant's research program to departmental initiatives and focus. All faculty recruited via the BSSP will be appointed at the Assistant Professor level.

APPLICATION INSTRUCTIONS: Please apply to the Scholars Program through the BSSP website at: (<http://www.med.umich.edu/medschool/research/bssp/>). A curriculum vitae (including bibliography), a three-page research plan, an NIH biosketch, and three original letters of support should all be submitted through the BSSP website. More information about the Scholars Program, instructions for applicants and those submitting letters of recommendation, and how to contact us is located on the BSSP web site: (<http://www.med.umich.edu/medschool/research/bssp/>). The deadline for applications is Friday, October 30, 2009.

The University of Michigan is an Affirmative Action/Equal Opportunity Employer.

GRADUATE PROGRAM

Don't just study great science. **LIVE IT.**

More information
www.hhmi.org/janelia

**Application deadlines
for Fall 2010 class**

Completed applications
will be reviewed as they
are received. Final dates
for completed applications
(including reference letters):

University of Chicago:
December 1, 2009

University of Cambridge:
March 1, 2010

HHMI
janelia farm
research campus

GRADUATE PROGRAM

The Janelia Farm Research Campus of the Howard Hughes Medical Institute is a new world-class facility near Washington, D.C. We are now accepting applications for a graduate program in collaboration with the University of Chicago and the University of Cambridge.

Following one year of work at Chicago or Cambridge, you will spend three or four years at Janelia Farm. You will join an interdisciplinary team of top scientists pursuing two challenging areas of research:

- Identifying the principles governing how groups of neurons process information
- Developing new imaging technologies and computational methods for image analysis

Think you have what it takes to join us?

WHERE THE BASIC SCIENCE

W
W



MEETS
THE

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OF MEDICINE

Submit your work to *Science Translational Medicine* today!

This fall, AAAS and *Science* will launch *Science Translational Medicine*, a new journal focused on applications of basic research knowledge that will improve human health.

The goal of *Science Translational Medicine* is simple: to help the scientific community harness decades of progress in research at the basic level and translate these biological discoveries into medical advances. Take this opportunity to have your work recognized in this groundbreaking new journal.

Papers in the following areas will be reviewed and considered for publication:

- Animal & Human Studies
- Applied Physical Sciences
- Behavior
- Bioengineering
- Biomarkers
- Cancer
- Cardiovascular Disease
- Cell Culture
- Chemical Genomics/Drug Discovery
- Data Mining
- Drug Delivery
- Gene Therapy/Regenerative Medicine
- Imaging
- Immunology/Vaccines
- Infectious Diseases
- Medical Informatics
- Medical Nanotechnology
- Metabolism/Diabetes/Obesity
- Neuroscience/Neurology/Psychiatry
- Pharmacogenetics
- Policy
- Toxicology & Pharmacokinetics
- And other interdisciplinary approaches to medicine



www.ScienceTranslationalMedicine.org

INTEGRATING MEDICINE AND SCIENCE

FACULTY POSITIONS IN CANCER BIOLOGY

Applications are invited for tenure-track faculty positions in the Cancer Biology and Genetics Program of the Sloan-Kettering Institute, Memorial Sloan-Kettering Cancer Center (www.ski.edu). Successful candidates will carry out independent research on the genesis, progression, prognosis, prevention and treatment of cancer that synergize with ongoing efforts at the Center. Areas of special interest are, but not limited to: cancer genetics, cancer stem cells, metastasis, tumor microenvironment, inflammation and cancer, and animal models of cancer.

New faculty members will join an interactive, interdisciplinary community of scientists and clinicians at the Center, which offers an outstanding basic and translational research environment within expanded state-of-the-art research facilities. Faculty will be eligible to hold graduate school appointments in the Gerstner Sloan-Kettering Graduate School of Biomedical Sciences, the Weill Cornell Graduate School of Medical Sciences of Cornell University, as well as the Tri-Institutional MD/PhD Training Program.

Cancer Biology & Genetics Faculty

Robert Benezra, PhD - Angiogenesis/Differentiation
Eric Holland, MD/PhD - Glioma Mouse Models
Anna Kenney, PhD - Neural Stem Cells/Brain Tumors
Robert Klein, PhD - Cancer Genetics
Johanna Joyce, PhD - Tumor Microenvironment
Joan Massague, PhD (Chairman) - Cell Regulation/Metastasis
Christine Mayr, MD, PhD - Oncogenic microRNA Target Control
Kenneth Offit, MD - Cancer Genetics
Harold Varmus, MD - Molecular Mechanisms of Oncogenesis
Andrea Ventura, PhD - microRNAs in Development and Cancer
Hans-Guido Wendel, MD - Genetic Basis for Drug Resistance

Candidates should e-mail their application, preferably in PDF format, to cancerbio@mskcc.org by November 1, 2009. The application should include a Curriculum Vitae, a description of past research, a description of proposed research (3-7 pages), and copies of three representative publications. Candidates should arrange to have three signed letters of reference sent by e-mail to cancerbio@mskcc.org. The letters should arrive by November 1, 2009. Inquiries may be sent to Maria Gordon at gordonm1@mskcc.org. EOE/AA.



Memorial Sloan-Kettering
Cancer Center
www.mskcc.org

CONFERENCE

BioJapan 2009 World Business Forum

Regeneration of Bioindustry

Great meeting spot with global mega pharma, unique biotech ventures, and academia with novel basic scientific research results. Completely free of charge matching system for one-on-one meeting pre-arrangement system is set in use. Nearly 250 "must-attend" seminars are also well-planned. Besides drug discovery area, such new theme like environment, food and cluster & venture, will be focused on this year. Join us and expand your business network at BioJapan2009. See you all in Yokohama!

October 7 to 9, 2009
10:00~17:00 Exhibition Hall

Venue
Organizer

Pacifico Yokohama
BioJapan Organizing Committee

Japan Bioindustry Association (JBA)
Japan Health Sciences Foundation (JHSF)
Society for Techno-innovation of Agriculture, Forestry and Fisheries (STAFF)
Japan Biological Informatics Consortium (JBIC)
Japan Association of Bioindustries Executives (JABEX)
Japan Pharmaceutical Manufacturers Association (JPMA)
NPO Kinki Bioindustry Development Organization (KBDO)
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CONFERENCE

HOWARD HUGHES MEDICAL INSTITUTE

JANELIA CONFERENCES SPRING 2010

The Janelia Farm Research Campus is pleased to announce its sixth season of conferences. These small, intense conferences are intended to foster rapid scientific advances and collaborative interactions. All participants are expected to contribute to the intellectual content of the meetings. The Howard Hughes Medical Institute fully supports the Janelia Conferences—there are no registration, accommodation, or dining fees for participants. The conference organizers invite all participants, selecting some from an open pool of applicants.

Imaging Transcription in Living Cells:

A Systems and Computational Approach • March 11-14, 2010

Organizers: Xavier Darzacq (École Normale Supérieure), Susan Gasser (Friedrich Miescher Institute for Biomedical Research), Robert Singer (Albert Einstein College of Medicine), and Robert Tjian (HHMI)

Structural Plasticity in the Mammalian Brain • March 21-24, 2010

Organizers: Tobias Bonhoeffer (Max Planck Institute of Neurobiology), Karel Svoboda, (Janelia Farm /HHMI), and Yi Zuo, University of California, Santa Cruz

The Neural Basis of Vibrissa-Based Tactile Sensation •

April 25-28, 2010

Organizers: Mitra Hartmann (Northwestern University), David Kleinfeld (University of California, San Diego), and Karel Svoboda (Janelia /HHMI)

Novel Approaches to Bioimaging II • May 2-5, 2010

Organizers: Mats Gustafsson and Tim Harris (Janelia Farm /HHMI), Jennifer Lippincott-Schwartz (National Institutes of Health), and Tony Wilson (University of Oxford)

Turning Images to Knowledge: Large-Scale 3D Image Annotation, Management and Visualization • May 9-12, 2010

Organizers: Michael Hawrylycz (The Allen Institute for Brain Science), B. S. Manjunath, (University of California, Santa Barbara), Maryann Martone (University of California, San Diego), and Fuhui Long, Gene Myers, and Hanchuan Peng (HHMI/Janelia Farm)

Form and Function of the Olfactory System • May 23-26, 2010

Organizers: Leslie Vosshall (HHMI/The Rockefeller University) and Kazushige Touhara, (University of Tokyo)

Janelia Farm offers scholarships to graduate students who would otherwise be unable to participate in the conferences. The scholarships fund students who are from groups that are underrepresented in the sciences or who come from disadvantaged backgrounds.

Information: www.hhmi.org/janelia

Application deadline: November 10, 2009

HHMI
janelia farm
research campus

POSITIONS OPEN

ASSISTANT PROFESSOR

The Department of Biology at Denison University invites applications for a tenure-track position beginning fall 2010. Ph.D. is required; a strong potential for excellence in teaching and a productive research program involving undergraduates are essential. Area of specialization within the broader scope of cellular, molecular, or physiological research is open. Teaching responsibilities include introductory courses for both majors and nonmajors, an intermediate level cellular and molecular biology course, and two advanced cellular, molecular, or physiological courses.

Denison University is a selective and nationally ranked, residential liberal arts college located in Granville, Ohio, 25 miles east of the Columbus metropolitan area. We are committed to fostering an academically and culturally diverse faculty and community. The Department of Biology currently has 12 full-time faculty members who represent a wide range of specialty areas, and the annual teaching load is two-one (each course with laboratory component). Our new Talbot Hall of Biological Science includes individual faculty offices and research laboratories, classrooms equipped for multimedia instruction, teaching laboratories, and greenhouses. We have departmental and college programs for mentoring and enhancing teaching. We offer competitive startup funds, a generous sabbatical leave program, a junior faculty leave following a successful third year review, and summer support for student and faculty research. See our website: <http://www.denison.edu/biology> for more detailed descriptions of the position and the program.

Applicants should submit electronic application materials online at website: <http://www.denison.edu/offices/humanresources>: a cover letter addressing their motivations for teaching at a small, undergraduate, residential, liberal arts college; separate statements of: (1) teaching philosophy along with brief descriptions of proposed advanced courses, (2) research interests and future plans, and (3) potential to foster and support diversity among our students, faculty, and community; curriculum vitae; copies of transcripts (graduate and undergraduate); and the names, e-mail addresses, and telephone numbers of three references. Review of applications will begin October 2, 2009, and continue until the position is filled.

In a continuing effort to diversify our campus community, we actively encourage applications from people of color, women, veterans, people of diverse sexual identities/orientations, and others who may contribute positively to the diversification of ideas and perspectives. For additional information and resources about diversity at Denison, please see our Diversity Guide at website: <http://www.denison.edu/offices/humanresources>. Denison University is an Affirmative Action/Equal Opportunity Employer.

PEDIATRICS, CELL BIOLOGY AND PHYSIOLOGY

Children's Hospital of Pittsburgh/University of Pittsburgh School of Medicine

Position is available to study the dynamic regulation of the endocytic trafficking of cystic fibrosis transmembrane conductance regulator by multiprotein interactions, including nonconventional molecular motors, Rab GTPases, and ubiquitin ligases. Immortalized human airway and intestinal epithelial cells, primary differentiated human bronchial epithelial cells among the model systems. Experience in molecular biology, cell biology, or electrophysiology necessary. *Must be an American citizen or permanent U.S. resident* (funded by the NIH award issued under the American Recovery and Reinvestment Act of 2009).

Send curriculum vitae and recent publications to: Agnes Swiatecka-Urban, M.D., F.A.S.N., Assistant Professor, Children's Hospital of Pittsburgh, Rangos Research Center, 7th Floor, Room 7119, Pittsburgh, PA 15201. Or e-mail: asurban@pitt.edu. The University of Pittsburgh is an Affirmative Action, Equal Opportunity Employer. Applications from women and minorities are encouraged.

POSITIONS OPEN

POSTDOCTORAL RESEARCH POSITIONS

Immediate openings due to cancellation

University of California, Irvine

School of Medicine

University of California, Irvine offers a Neuroscience Epilepsy Research Postdoctoral Program Fellowship. The multidisciplinary postdoctoral program in epilepsy research has multiple openings for non-tenured, academic term appointments as Postdoctoral Scholar. The program supports diverse approaches to the understanding of the fundamental neurobiological processes leading to epilepsy and/or holding promise for its cure. Participating laboratories include: **Tallie Z. Baram, Ph.D., M.D.**: Mechanisms of epileptogenesis after febrile seizures, regulation of ion channels, in vivo and in vitro imaging; **Devin Binder, M.D.**: Water transport and water channels in epilepsy, Aquaporins, optical imaging; **Steven Cramer, M.D.**: Functional imaging and robotics for identification and cure of excitotoxic and ischemic insults; **Christine M. Gall, Ph.D.**: Neurotrophins, integrins, activity-dependent plasticity; **Alan L. Goldin, M.D., Ph.D.**: Sodium channels, transgenic approaches, electrophysiology; **Gary Lynch, Ph.D.**: Regulation of excitability and synaptic function and plasticity; **Charles E. Ribak, Ph.D.**: Granule cell plasticity, neuroanatomy; **Mick Rugg, Ph.D.**: Functional imaging of learning and memory circuits in health and disease; **Ivan Soltesz, Ph.D.**: Electrophysiology, computational neurobiology, interneurons and inhibition; **Martin Smith, Ph.D.**: Novel signaling - Agrin and neuronal excitability; **Oswald Steward, Ph.D.**: Mechanisms of vulnerability to excitotoxicity; **John Weiss, M.D., Ph.D.**: Excitotoxicity, calcium, and zinc trafficking. See website: <http://www.ucihs.uci.edu/epilepsyresearch/>.

These positions starting summer/fall 2009 are funded by an NIH training grant (T-32); *eligible candidates must be U.S. citizens or noncitizen nationals or must be lawfully admitted for permanent residence*. An M.D. or Ph.D. degree is required, and M.D.-qualified candidates are highly encouraged to apply. Salary is commensurate with experience and based on the Kirschstein-National Research Service Award postdoctoral stipend levels for 2009. Candidates should submit resume and references electronically to e-mail: sara.johnson@uci.edu; Postdoctoral Search/Epilepsy, c/o Department of Anatomy and Neurobiology, Med Surge II, Room 364, University of California, Irvine, Irvine, CA 92697-1275.

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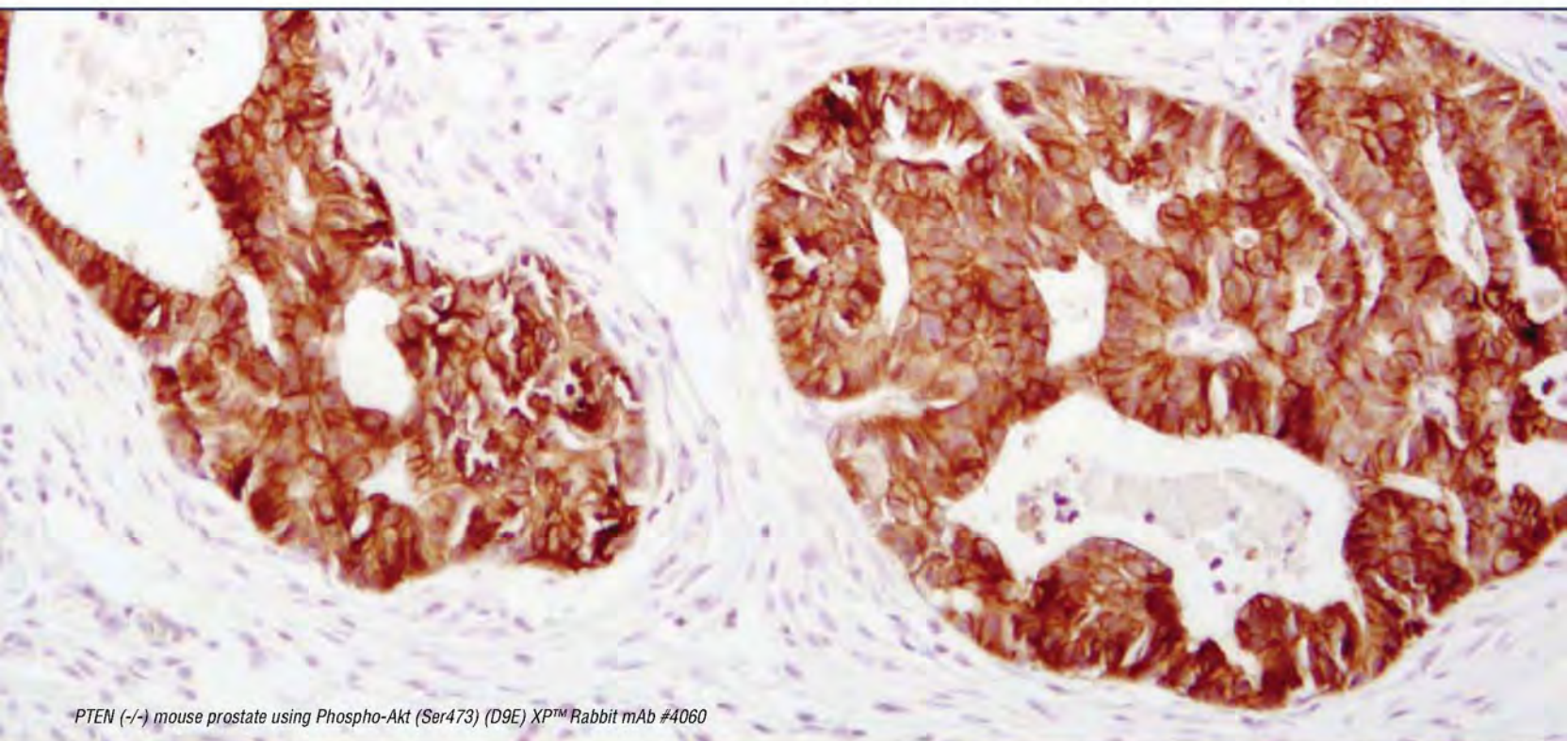
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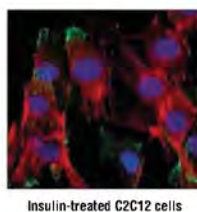
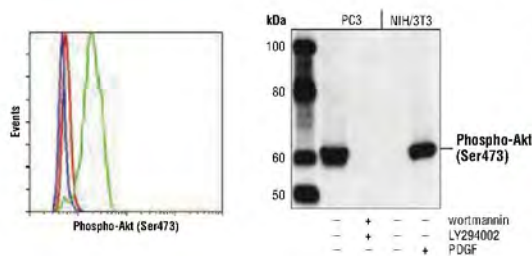
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